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Preparation and Characterization of Lyophilized Cyclodextrin Complexes of a Hemisuccinate Ester of Delta-9-Tetrahydrocannabinol for Transmucosal Delivery

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PREPARATION AND CHARACTERIZATION OF LYOPHILIZED CYCLODEXTRIN COMPLEXES OF A HEMISUCCINATE ESTER OF DELTA-9-TETRAHYDROCANNABINOL FOR TRANSMUCOSAL DELIVERY

A Dissertation

Presented in partial fulfillment of requirements for the degree of

Doctor of Philosophy

in the Department of Pharmaceutics

The University of Mississippi

by

SAMPADA B UPADHYE

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ABSTRACT

Δ^9-Tetrahydrocannabinol (THC) is the primary active ingredient of the plant *Cannabis sativa* (marijuana), responsible for the majority of the pharmacological effects. The most promising clinical applications of THC approved by the Food and Drug Administration (FDA) are for the control of nausea and vomiting associated with chemotherapy and for appetite stimulation of AIDS patients suffering from anorexia and wasting syndrome. The only dosage form currently approved by FDA is an oral, soft gelatin capsule (e.g. Marinol®). In addition, orally administered THC from Marinol® has shown slow and variable absorption due to low oral solubility and first pass metabolism. Our strategy towards developing THC transmucosal formulations involved development of hydrophilic prodrugs of THC and solubilization and stabilization of prodrugs in oral formulations. In the present study, the hemisuccinate ester of THC (THC-HS) has been investigated for its potential to form inclusion complexes with modified synthetic beta-cyclodextrins (CDs). The formation of 1:1 inclusion complexes of THC-HS with random methylated beta-cyclodextrin (RAMEB) and 2-hydroxypropyl beta-cyclodextrin (HPBCD) was demonstrated by an A_L type curve with the slopes less than unity by the phase solubility method.

We evaluated the effect of RAMEB and HPBCD on chemical and enzymatic stability and *in vitro* permeation across excised buccal mucosa of THC-HS. There was a significant reduction in chemical hydrolysis of complexed prodrug as compared to free prodrug. RAMEB afforded better stability profile and lower degradation rate constants as compared to HPBCD at all the pHs

tested and in enzymatic conditions too. *In vitro* permeation experiments demonstrated almost 63-fold increase in the permeability of THC-HS across excised buccal mucosa, in the presence of RAMEB as compared to the surfactants. Lyophilized solid dispersions of THC-HS with RAMEB and HPBCD were evaluated for their stability and drug release characteristics. RAMEB proved superior to HPBCD in enhancing the stability of THC-HS in solid dispersions as well as the rate of release of the prodrug from the solid dispersions. Finally design of experiments approach was implemented by proposing a 2^3 factorial design to identify critical formulation variables required in the development of transmucosal polymeric films of THC-HS with enhanced solubility and stability.

DEDICATION

Dedicated To My Parents

Mrs. Sulabha B Upadhye & Mr. Bhaskar J Upadhye

To Whose Vision And Tireless Encouragement,

I Owe All My Success And Good Life Here

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CHAPTER 1

INTRODUCTION

1.1. Δ^9-Tetrahydrocannabinol

The use of herbal marijuana in medicinal applications like multiple sclerosis and alzheimer's disease in now being widely discussed in both the medical and lay literature. Ballot initiatives in California and Arizona have made medical marijuana legal under certain circumstances. Most recently, the *California Proposition 19* which sought to legalize various marijuana-related activities in California (although not as a matter of federal law), allowing local governments to regulate these activities, permitting local governments to impose and collect marijuana-related fees and taxes, and authorizing various criminal and civil penalties was defeated.

Marijuana has been widely used for hundreds of years as an addiction aid or herbal remedy. Pure Δ^9-tetrahydrocannabinol (THC) is the one of the major active constituent out of 66 constituents of *cannabis sativa*. The crude marijuana, an undefined herb containing approximately 480 substances has not been approved by the US Food and Drug Administration for use as a medicine.[1,2]

THC is the main source of the pharmacologic effects of cannabis and most of its effects are mediated through agonistic action cannabinoid (CB) receptors. To date, two subtypes of these receptors have been identified, the CB1 receptor (cloned in 1990) and CB2 receptor (cloned in 1993), both coupled through inhibiting G proteins (Gi proteins), negatively to adenylate cyclase and positively to mitogen- activated protein kinase. Activation of Gi proteins causes inhibition of adenylate cyclase, thus inhibiting the conversion of AMP to cyclic AMP. A total of 66 phytocannabinoids have been identified, most of them belonging to several subclasses or types: the cannabigerol CBG), cannabichromene (CBC), cannabidiol (CBD), Δ^9-THC, Δ^8-THC, cannabicyclol (CBL), cannabielsoin (CBE), cannabinol (CBN), cannabinodiol(CBDL) and

cannabitriol (CBTL) types. The cannabinoid acids of Δ^9-THC, CBD, CBC and CBG are the quantitatively most important cannabinoids present in the plant. The cannabinoid acids of THC are devoid of psychotropic effects and have to be decarboxylated to the phenols to produce marijuana-like effects, e.g. by smoking the dried plant matter. [3]

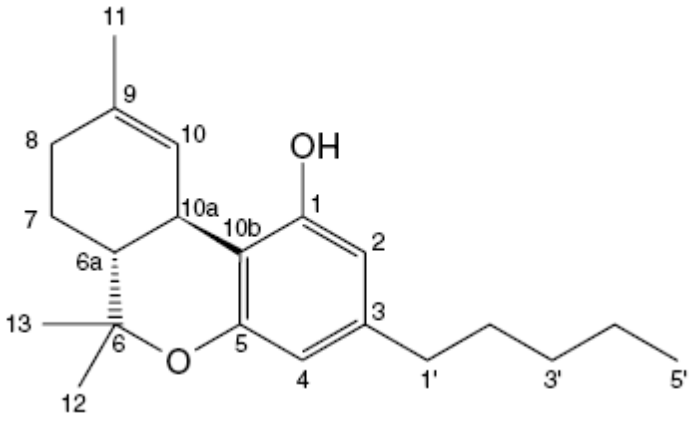

Figure 1-1 Structure of Δ^9-THC

Natural Δ^9-THC has two chiral centres at C-6a and C-10a in the *trans* configuration. Usually the acronym THC is applied to this naturally occurring (–)-*trans*-isomer of Δ^9-THC, and will be used in this text as well. The generic name for Δ^9-*trans*tetrahydrocannabinol is dronabinol. Marinol™ (Unimed Pharmaceuticals, Inc.) contains synthetic dronabinol, dissolved in sesame oil, as capsules of 2.5, 5 and 10mg of dronabinol.

1.2. Physicochemical Properties and Degradation of THC

THC and many of its metabolites are highly lipophilic and essentially water-insoluble.[4] Calculations of the n-octanol/water partition coefficient (K_{ow}) of THC at neutral pH vary between 6000 using shake-flask methodology.[5] The wide range for aqueous solubility and K_{ow}, can be attributed to the difficulty of uniformly dissolving this essentially water-insoluble

substance and accurately measuring small amounts of it. The spectrophotometric pKa is 10.6.[4] THC is thermolabile and photolabile.[6,7] Storage leads to a cumulative decrease in THC content through oxidation of THC to CBN.[8,9] THC rapidly degrades in acid solutions. The kinetics seem to be first order and specifically hydrogen ion-catalysed so that significant degradation is assumed to occur in the normal stomach with a half-life of 1 hour at pH 1.[4] Decarboxylation of the THC acids to the corresponding phenols occurs readily over time, upon heating or under alkaline conditions.[8,9] Heating for 5 minutes at a temperature of 200–210°C has been reported to be optimal for this purpose, but a few seconds in burning cannabis cigarettes are equally sufficient. Slow decarboxylation of THC acid occurs at room temperature.

1.3. Pharmacokinetics of Δ^9-Tetrahydrocannabinol

Cannabis products are commonly either inhaled by smoking a cannabis cigarette, taken orally as dronabinol capsules or in baked foods or liquids. Various other routes of administration and delivery forms have been tested for therapeutic purposes. The rectal route with suppositories has been applied in some patients,[10] and dermal[11]

and sublingual[12] administration are under investigation. Other methods include eye drops to decrease intraocular pressure,[13] as well as aerosols and inhalation with vaporisers to avoid the harm associated with smoking.[14,15] The kinetics of cannabinoids are much the same for females and males,[16] as well as for frequent and infrequent users.[17,18]

1.4. Therapeutic indications of THC

Several pharmacological effects of THC may well be clinically useful. One of the most prominent therapeutic uses of THC is its anti-emetic effect in cancer chemotherapy. Another is

the ability to lower increased intraocular pressure, as occurs in glaucoma. Another potential use of THC is as a bronchodilator in the treatment of patients with bronchitis and asthma. There are a variety of actions on the central nervous system associated with THC administration for therapeutic purposes. A syndrome of feelings described as a "high" often occurs. This euphoria includes such effects as elation, heightened awareness, giddiness and some distortions of activities and interactions with other people. Some researchers have found that the production of a "high" correlates well with its anti-emetic activity. Unpleasant effects on the central nervous system have also been reported, including visual hallucinations and severe, continuous depersonalization.

By far, most research on THC has involved the use of oral THC (dronabinol) for the treatment of nausea and vomiting in cancer chemotherapy as well as appetite loss in AIDS wasting syndrome.

1.5. Cannabinoid therapy: Design of formulation systems for various routes of administration

There have been number of attempts to develop formulation systems for administration via oral, sublingual transdermal routes for the USFDA approved indication inc ancer chemotherapy.

1.5.1. Oral administration of cannabinoids

Synthetic THC is known as *dronabinol*. It is available as a prescription drug (under Marinol® in several countries including the United States and Germany. In the United States, Marinol is a Schedule III drug, available by prescription, considered to be non-narcotic and to

have a low risk of physical or mental dependence. Marinol has been approved by the U.S. Food and Drug Administration (FDA) in the treatment of anorexia in AIDS patients, as well as for refractory nausea and vomiting of patients undergoing chemotherapy. However, the reduced bioavailability of orally administered THC, due to low absorption and high first pass metabolism, prompts the development of more reliable administration forms such as THC solutions for inhalation, sublingual or transdermal films.

1.5.2. Transdermal administration of cannabinoids

The skin permeation behavior of THC, cannabidiol and cannabinol has been investigated in several experimental studies. The transdermal administration increases the duration of action compared with all the other routes of application. This is advantageous in users requiring control of long-term pain and other applications with continuous effects. Since cannabinoids are highly lipophilic molecules, they accumulate within the upper skin layer (Stratum corneum) and permeate slowly to lower strata. Hence their effective subcutaneous administration requires permeation enhancement. In a study by Touitou *et al*,[19] which used the more stable Δ^8 THC isomer, the permeability coefficient of THC was significantly enhanced from the formulations containing oleic acid in propylene glycol/water/ethanol. Significant THC concentrations were found in the blood of hairless rats treated with formulations containing THC 26.5 mg/g. In several *in vitro* experiments carried out by Stinchcomb *et al*,[11] mean flux rates for Δ^8-THC, cannabidiol and cannabinol through samples ranged from 0.73 to 4.67 nmol/cm^2/h. They used cannabinoid solutions in propylene glycol/water/ethanol mixtures of various concentrations. An ethanol concentration of 30-33% gave the highest flux rates. Based on a THC clearance of 14 L/h and an average plasma level of 10 ng/ml, a required therapeutic delivery rate of THC of

about 9 nmol/cm^2/h can be estimated which is somewhat closer to that achieved in the studies by Stinchcomb and colleagues.

A transdermal delivery system for cannabidiol involving the use of ethosomal carriers has also been designed. Ethosomes are malleable vesicles tailored for enhanced delivery of active agents. They are composed of phospholipids, ethanol, and water and encapsulate and deliver, through the skin, highly lipophilic molecules such as cannabinoids, testosterone, and minoxidil, as well such as propranolol and trihexyphenidil. *In vivo* administration of ethosomal cannabidiol to nude mice produced significant amounts of drug in the skin and in the underlying muscle.[20] Valiveti et al developed a transdermal therapeutic system to deliver Δ^8-THC. The plasma concentration of Δ^8-THC reached a mean steady-state level of 4.4 ng/ml within 1.4 h and was maintained for at least 48 h.[21]

1.5.3. Sublingual administration of cannabinoids

Sativex® is a cannabis-based pharmaceutical product containing Δ^9-tetrahydrocannabinol (THC) and cannabidiol (CBD) in a 1:1 ratio, delivered in an oromucosal (mouth) spray. Each spray of Sativex delivers a fixed dose of 2.7mg THC and 2.5mg CBD. It has been approved as adjunctive treatment for neuropathic pain in patients with multiple sclerosis (MS) in UK and Canada. It is being investigated for the management of other MS symptoms, such as spasticity. THC:CBD spray is regulated as a narcotic. Five randomized controlled trials (RCTs) compared the benefits and harms of THC:CBD spray with placebo. A total of 368 patients with various neurological conditions (including MS) were recruited. In some trials, THC:CBD spray significantly reduced neuropathic pain, spasticity, muscle spasms and sleep disturbances. The most common adverse events (AEs) reported in trials were dizziness, sleepiness, fatigue, feeling

of intoxication and a bad taste. Long-term safety and the potential for dependence, abuse, misuse and diversion are unknown.[12]

1.5.4. Buccal administration of cannabinoids

Despite the promising clinical potential of THC, an effective dosage form for delivery of THC has not been developed to date. This is mainly attributed to its pharmacokinetic limitations and poor physicochemical properties. THC is a poorly water-soluble, amorphous substance which is sticky, resin-like and highly viscous making it difficult to handle and process. Furthermore, THC is an unstable drug. In acidic environment and in presence of heat, it undergoes hydrolysis to yield cannabidiol and in presence of air it undergoes oxidation producing cannabinol as the degradation product. Degradation due to visible light is also reported.[8] The prodrug approach has been the most successful strategy in improving the pharmaceutical, pharmacokinetic, and pharmacodynamic properties of drugs. Prodrugs are pharmacologically inactive derivatives of active drugs designed to maximize the amount of active drug that reaches its site of action, through manipulation of the physicochemical, biopharmaceutical or pharmacokinetic properties of the drug.[22-27]

Prodrugs are converted into the active drug within the body through enzymatic or non-enzymatic reactions. To overcome the pharmacokinetic limitations and improve the physicochemical properties of the parent drug, several prodrugs of THC have been developed and investigated. These include THC-hemisuccinate (THC-HS), THC-hemiglutarate (THC-HG).[28] Several researchers have studied the stability and bioavailability of THC-HS in various dosage forms. Elsohly *et al* have investigated the possibility of delivering THC-HS via the rectal route of administration. Studies carried out using THC-HS in a lipophilic base in dogs exhibited approximately 64 % bioavailability. [29]Munjal *et al* have investigated the possibility of delivering

the prodrug via oral transmucosal route. The authors studied the stability of THC-HS when incorporated into poly (ethylene oxide) matrices via hot-melt casting methods.[30,31] THC-HG is another prodrug of THC which was developed to overcome the solubility and stability issues associated with THC. The formulation development of THC-HG transmucosal films has been studied by Thumma et al where the main focus was the stabilization of THC-HG in hot-melt polymeric matrices.[32,33]

1.6. Transmucosal Delivery of cannabinoids

Due to the significant limitations associated with traditional routes of administration, several non-parenteral routes such as nasal, ocular and transdermal have also been explored for the systemic delivery of THC. While each of these routes has its own disadvantages, the oral transmucosal route has several advantages. The intra-oral mucosal route has easy accessibility, an expanse of smooth muscle and relatively immobile mucosa and hence is suitable for administration of retentive dosage forms. Drugs administered by this route bypass the first pass metabolism and gain direct access to the systemic circulation through the internal jugular vein leading to a high bioavailability. Oral mucosal delivery is non-invasive and less intimidating for patients as compared to other routes of administration (e.g. intravenous and intra muscular). The oral cavity serves as a useful site for delivery for patients suffering with nausea or vomiting, or in a state of unconsciousness, with an upper gastrointestinal tract disease or surgery which affects oral absorption, or those who have difficulty swallowing the peroral medications. Other advantages include low enzymatic activity, suitability for drugs that are susceptible to acid hydrolysis in the stomach, painless administration, easy drug withdrawal in case of toxicity,

facility to include permeation enhancer/enzyme inhibitor or pH modifiers in the formulation and versatility in designing as multidirectional or unidirectional release

systems for local or systemic actions. The small surface area, salivary wash out of the dissolved drug and barrier property of buccal mucosa stand as the major limitations in the development of buccal adhesive drug delivery systems. However, these limitations can be overcome by tailoring the formulation to include permeation enhancers and bioadhesive polymers.

1.7. Problems associated with the prodrug approach

The other problems associated with transmucosal oral delivery of THC prodrugs are: a) high instability to heat and hydrolysis and prone to oxidation, b) the poor solubility of the prodrug (better than the parent drug) making its absorption, dissolution rate- limited, and c) since the prodrug is sticky and resinous in nature, handling issues are a major concern in formulation of the drug. Hence, suitable methods need to be employed to make it free-flowing. At this stage, a solubilization approach might be needed to enhance the solubility of THC-HS and maintain a higher concentration in the gastrointestinal tract (GIT) in order to enhance absorption. Earlier, attempts have been made by Repka *et al* to enhance the solubility and stability of THC-HS and THC-HG using different polymers, solubilizers, anti-oxidants and pH modifiers. These solubilization methods were only moderately successful in increasing the solubility of the prodrugs warranting a better solubilization strategy. One of the most commonly explored technologies to enhance the solubility and, in turn, the absorption of water-insoluble drug molecules from the GIT is the use of cyclodextrin complexation of drugs. [34]

1.8. Cyclodextrins as solubilizing agents for enhanced oral bioavailability

Cyclodextrins have been used extensively in pharmaceutical research and development, and there are currently over 30 marketed cyclodextrin-containing pharmaceutical products worldwide.[34] Cyclodextrins possess a unique ability to complex with drugs enabling them to increase solubility, reduce bitterness, enhance stability, and decrease tissue irritation upon dosing. One of the most common applications of cyclodextrins cited in the pharmaceutical literature is the enhancement of solubility and in turn increasing the bioavailability of the drug. Cyclodextrins are cyclic oligosaccharides composed of 6-8 dextrose units (α-, β-, γ-cyclodextrins, respectively) joined through 1-4 bonds.[35] Because the interior of these molecules are relatively hydrophobic and the exterior relatively hydrophilic, they tend to form inclusion complexes of the type illustrated by Figure 1-2.

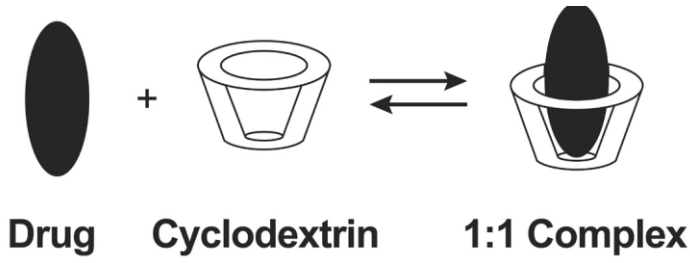

Figure 1-2. Equilibrium binding of a drug with a cyclodextrin

The driving forces for inclusion complexation are both enthalpic and entropic in nature and not fully understood. Although the ability of a drug molecule to "fit" into the cyclodextrin torus is critical with the 6.0-6.5 Å opening of β-cyclodextrin being optimal for many biomedically relevant molecules, "fit" by itself is not the only consideration. In forming inclusion complexes, the physical and chemical properties of both the drug molecule and the cyclodextrin molecule can be altered. Inclusion complexation has been used to solubilize,

stabilize and decrease the volatility of drug molecules. Additionally, it has been used to ameliorate the irritancy and toxicity of drug molecules. It might be argued that other techniques can achieve these same goals, so why use cyclodextrins? Most other excipients used to solubilize and stabilize drugs do so because of changes in the bulk properties of the resultant solvent. For example, co-solvents like various alcohols and glycols will increase the solubility of a poorly water soluble drug in a non-linear fashion with respect to co-solvent concentration. As illustrated in Figure 1-2, most drugs form 1:1 complexes with various cyclodextrins. This complexation can be defined by a binding constant $K_{1:1}$ and equation 1, where $[Drug]_{complex}$ represents the concentration of drug in the complex form, $[Drug]_{free}$ represents the free drug concentration and $[cyclodextrin]_{free}$ represents the concentration of free cyclodextrin.

$$K_{1:1} = \frac{[Drug]_{complex}}{[Drug]_{free}[Cyclodextrin]_{free}} \qquad (1)$$

In solubility considerations, if $[Drug]_{free}$ represents the solubility of a drug in the absence of a cyclodextrin, then the increase in solubility of a drug is largely driven by the product of $K_{1:1}$ and $[Cyclodextrins]_{free}$, with $[Cyclodextrin]_{free}$ equal to $[Cyclodextrin]_{total}$ minus $[Drug]_{complex}$. Since most values of $K_{1:1}$ fall within the range of 100-20,000M^{-1} and $[Cyclodextrin]_{total}$ usually is less than 0.1-0.2 M, the maximum increases in solubility that can be expected from 1:1 interactions with a cyclodextrins are in the range of 1,000 – 2,000. [36]

It is important to realize that the kinetics of inclusion complex formation and dissociation between a cyclodextrin and a drug molecule is fast. Where these events have been measured by various perturbation and competitive binding techniques, the half-lives for formation/dissociation are much less than one second and occur at rates very close to diffusion controlled limits with the complexes being continually formed and broken down. Inclusion complexation is a very

dynamic process and in no way does the complex species resemble a covalent species. For weakly bound drugs, dilution is sufficient to account for rapid and complete dissociation. For highly bound drugs, displacement of drugs by competing agents like plasma proteins and cholesterol may contribute to dissociation of the complex.

1.8.1. Study of CD Complexation and Dilution Effects

The most widely used approach to study inclusion complexation (Figure 1-3) is the phase solubility method described by Higuchi and Connors,[37] which examines the effect of a solubilizer, i.e, CD or ligand, on the drug being solubilized, i.e, the substrate. Phase solubility diagrams are categorized into A and B types; A type curves indicate the formation of soluble inclusion complexes while B type suggest the formation of inclusion complexes with poor solubility. A B_S type response denotes complexes of limited solubility and a B_I curve indicates insoluble complexes. A-type curves are subdivided into A_L (linear increases of drug solubility as a function of CD concentration), A_P (positively deviating isotherms), and A_N (negatively deviating isotherms) subtypes. β-CD often gives rise to B-type curves due to their poor water solubility whereas the chemically modified CDs like HP-β-CD and SBE-β-CD usually produce soluble complexes and thus give A-type systems.

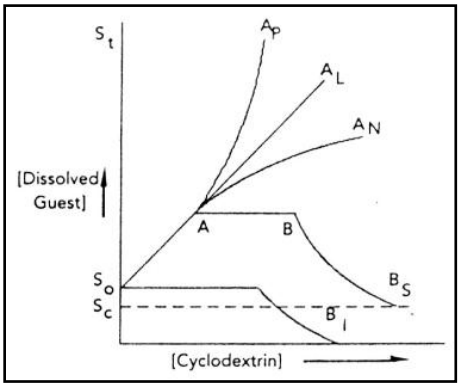

Figure 1-3: Phase solubility diagram

In the case of a 1:1 complex, using the following equation one can determine the equilibrium binding or association constant, *K*, from the slope of the linear portion of the curve.

$$K_{a:b} = slope / S_0 (1 - slope) \qquad (2)$$

where S_o is the intrinsic solubility of the drug studied under the conditions.

1.8.2. Modified Cyclodextrins

Two of the parent cyclodextrins, α- and β-cyclodextrin, are known to be parenterally unsafe due to severe nephrotoxicity. The etiology of the nephrotoxicity of both α- and β-cyclodextrin is unknown but appears to be related to either cyclodextrin uptake by the kidney tubule cells followed by disruption of intracellular function, or the extraction of lipid membrane components by the cyclodextrin. Modification of the parent cyclodextrins to improve the safety, while maintaining the ability to form inclusion complexes with various substrates, has led to the synthesis of random methylated β-cyclodextrin (RAMEB), 2-hydroxyprpoyl- β-cyclodextrin (HPBCD), sulfobutyl ether- β-cyclodextrin (SBECD) etc. These synthetically modified cyclodextrins have better solubility and excellent safety profile than the parent cyclodextrins. They have been used in parenteral formulations, oral, transdermal and ocular formulations. Synthetic cyclodextrins like RAMEB, HPBCD and SBECD are approved by regulatory agencies in USA and Europe. [35,38]

1.8.3. Factors Influencing Inclusion Complex Formation

Type of CD can influence the formation as well as the performance of drug/CD complexes.[39-42] For complexation, the cavity size of CD should be suitable to accommodate a drug molecule of particular size.[42-44] Compared with neutral CDs, complexation can be better when the CD and the drug carry opposite charge but may decrease when they carry the same

charge.[45,46] In general, ionic forms of drugs are weaker complex forming agents than their nonionic forms,[47] but in the case of mebendazole, the un-ionized form was less included in HP-β-CD than the cationic form.[48]

Temperature changes can affect drug/CD complexation. In most cases, increasing the temperature decreased the magnitude of the apparent stability constant of the drug/CD complex and the effect was reported to be a result of possible reduction of drug/CD interaction forces, such as van der Waals and hydrophobic forces with rise of temperature.[46,49] However, temperature changes may have negligible effect when the drug/CD interaction is predominantly entropy driven (ie, resulting from the liberation of water molecules hydrated around the charges of guest and host molecules through inclusion complexation).[45]

Method of preparation, viz co-grinding, kneading, solid dispersion, solvent evaporation, co-precipitation, spray drying, or freeze drying can affect drug/CD complexation. The effectiveness of a method depends on the nature of the drug and CD.[44,50,51] In many cases, spray drying,[52,53] and freeze drying were found to be most effective for drug complexation.[54,55]

When added in small amounts, water-soluble polymers or ion pairing agents enhance CD solubilizing effect by increasing the apparent complex stability constant. The polymers or ion pairing agents due to their direct participation in drug complexation, improve both pharmaceutical and biological properties of drug/CD complexes, independent of drug's physiochemical properties.[56-70] Certain additives may compete with drug molecules for CD cavities and thus decrease the apparent complex stability constant, eg, additives with positive and negative hydrotropic effect.[71,72] Though water structure forming agents added to CD solutions generally increase the total drug solubility, they showed opposite effects with

clotrimazole.[73] Simultaneous complexation and salt formation with hydroxy carboxylic acid (HA) significantly increased the CD solubilizing power for a sparingly water-soluble amine type drug by forming drug/CD/HA multicomponent systems.[60,74] Co-solvents can improve the solubilizing and stabilizing effects of CDs, eg, use of 10% propylene glycol in development of an oral itraconazole preparation containing 40% of HP-β-CD.[75] Sometimes co-solvents may hinder drug complexation by competitive inclusion, eg, presence of 10% propylene glycol decreased the solubilizing effect of HP-β-CD for itraconazole. On dilution, the presence of propylene glycol favored absorption and precipitation of itraconazole in GI fluids and formulation by providing increased percentage of the free drug. The increased percentage of the free drug in presence of co-solvent was reported to be a result of lesser intrinsic solubility of the drug compared with the dilution concentration line at a given HP-β-CD concentration.[76]

1.8.4. Effect on Drug Bioavailability

CDs enhance the bioavailability of insoluble drugs by increasing the drug solubility, dissolution, and/or drug permeability. CDs increase the permeability of insoluble, hydrophobic drugs by making the drug available at the surface of the biological barrier, eg, skin, mucosa, or the eye cornea, from where it partitions into the membrane without disrupting the lipid layers of the barrier. In such cases it is important to use just enough CD to solubilize the drug in the aqueous vehicle since excess may decrease the drug availability.[77] At low RAMEB concentrations, when hydrocortisone was in suspension, increasing the CD concentration increased the drug flux. At higher CD concentrations, when the drug was in solution, increasing the CD concentration decreased the flux. [77] It was found that addition of polymers can further enhance the drug permeability from aqueous CD solutions. Carboxy methyl cellulose (CMC) enhanced triclosan bioavailability from toothpastes containing β-CD by forming a

drug/CD/CMC complex with improved substantivity.[78] CDs increased the bioavailability of lipophilic itraconazole from both an oral solution and an intravenous formulation by improving the drug solubility and absorption.[79]

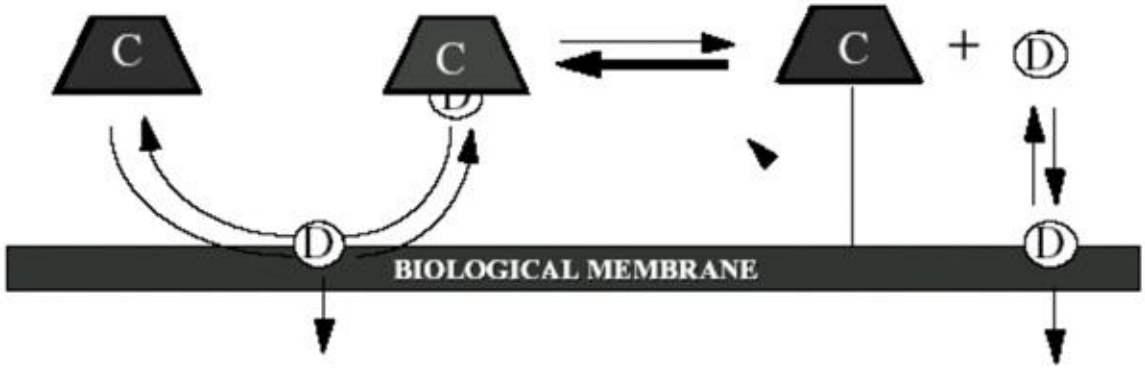

Figure 1-4: Penetration enhancement by CD's through biological membrane

1.8.5. Effect on Drug Stability

CDs can improve the stability of several labile drugs against dehydration, hydrolysis, oxidation, and photodecomposition and thus increase the shelf life of drugs. It was reported that CD-induced enhancement of drug stability may be a result of inhibition of drug interaction with vehicles and/or inhibition of drug bioconversion at the absorption site.[80] By providing a molecular shield, CD complexation encapsulates labile drug molecules at the molecular level and thus insulates them against various degradation processes. SBE-β-CD showed greater stability enhancement of many chemically unstable drugs than other CDs.

Since the hydrolysis of drugs encapsulated in CDs is slower than that of free drugs,[81] the stability of the drug/CD complex, ie, the magnitude of the complex stability constant, plays a significant role in determining the extent of protection. Very low concentrations of HP-β-CD (1% or lower), due to formation of a more physically unstable complex, did not protect taxol as

effectively as higher CD concentrations. The effect of complexation on drug stability can be represented by the following equation

$$1/k_0 - k_{obs} = 1/K_c\,(k_0 - k_c)\,[CD] + 1/(k_0 - k_c) \quad (3)$$

where k_o is the degradation rate constant of free drug, k_{obs} is the observed degradation rate constant in the presence of CD, k_c is the degradation rate constant of the drug within CD, K_c is the stability constant for the complex, and [*CD*] is the concentration of CD.[82]

1.8.6. CD Applications in Oral Drug Delivery

CDs enhance the mucosal drug permeability mainly by increasing the free drug availability at the absorptive surface.[83] CD complexation can provide better and uniform absorption of low-soluble drugs with poor and erratic absorption and also enhance the drug activity on oral administration. CD complexation increased the absorption of poorly water-soluble drugs, delivered via buccal or sublingual mucosa.[84,85] Complexation of miconazole, econazole, and clotrimazole with HP-β-CD and genuine CDs increased the toxicity of these drugs on a human buccal cell culture model (TR_{146}) by causing drug supersaturation.[85] Complexation can also mask the undesirable taste of drugs. Complexation with CDs suppressed the bitter taste of oxyphenonium bromide. With the assumption that only the free drug molecule exhibits bitter taste, the extent of the suppression was reported to be dependent on the availability of free drug, regardless of the kind and concentration of CD.[86]

By the virtue of being able to form inclusion complexes with the drugs, CDs are known to enhance the solubility and protect the ester linkage of the compounds from hydrolysis. A vast amount of literature is published supporting the enhancement of bioavailability for oral formulations due to the inclusion complex formation with CDs. However, in very few cases the

absorption was hindered due to CD binding to the free drug and thus reducing its free concentration. In the process of inclusion complexation, the drug and CD are in dynamic equilibrium with each other leading to the inhibition of precipitation upon dilution up to a certain threshold. This phenomenon leads to the formation of supersaturated solutions of drug and CDs in the GIT with an enhanced absorption profile. In addition, the drugs can be lyophilized with the modified CDs to yield free-flowing powders. As a result, it was determined worthwhile to explore these advantages offered by CDs to develop an oral formulation for this ester prodrug of THC (THC-HS). The range of effects produced by CD complexation on absorption of water-insoluble molecules is variable due to the chemical nature of the drugs, dose and drug: CD ratio. Hence it is difficult to predict *a priori* or create a generalized model of efficacy of CD with every drug molecule. Yet, it is important to understand the physical and chemical interactions of CDs with the individual drug molecule to predict the efficacy of the complexation process on that particular drug. Lyophilized solid dispersions CDs would be incorporated into polymeric transmucosal films where CDs were expected to increase the soluble fraction of free drug THC-HS at the interface of the buccal membrane. Free THC-HS can then cross the buccal membrane into the plasma thus enhancing the bioavailability of THC through buccal route

1.9. Transmucosal Drug Delivery Systems

Historical development of drug delivery through the oral cavity

Historically, per-oral delivery has been the favored route of administration for the majority of therapeutic agents targeting systemic delivery. Oral administration generally leads to "transmucosal" absorption in the GI tract; however, this enteral route of delivery subjects compounds to extensive pre-systemic elimination, which may include GI degradation, metabolism or first pass clearance via the liver. This biotransformation has often resulted in low systemic bioavailability, short duration of therapeutic activity, and/or formation of inactive, and at times toxic metabolites.[87] Parenteral routes, such as intravenous (IV) or intramuscular (IM) administration, unlike oral delivery, permit therapeutic agents to gain direct entry into the systemic circulation and therefore reach the intended site of action more rapidly (with total bioavailability achieved in many IV cases). Unfortunately, such a mode of drug administration entails certain health risks, requires specialized equipment, often requires close medical supervision of the medication or may even necessitate the hospitalization of patients during treatment.

Systemic transmucosal delivery of therapeutic agents via the epithelium lining of accessible body cavities such as the oral cavity (mouth), nose, rectum and vagina have received renewed interest within the last decade. These routes have numerous advantages over their per-oral counterpart such as bypassing hepatic first pass clearance and therefore potentially improving systemic bioavailability. In addition, these transmucosal routes may eliminate the disadvantages of IV and IM administration. As a result, nasal[88-92], rectal[93-96] and vaginal[97,98] routes of delivery have gained the attention of many researchers.

The intraoral drug delivery sites are distinguished on the basis of their placement on the particular area in the oral mucosa. Two main type of transmucosal drug delivery routes are:

1. Buccal: Placed on inner side of the cheek mucosa or Gum area on the teeth
2. Sublingual : placed on the sublingual mucosa

Although the rectal, vaginal and ocular mucosa possess various advantages to drug delivery, poor patient acceptability associated with these sites makes them feasible at best for local drug delivery. The oral cavity, however, is a highly accepted route for both local and systemic drug delivery.[99] Indeed, transmucosal delivery via the oral mucosa is increasingly being considered to be the preferred route for many drug classes.

Technological advances in biomaterials and techniques have resulted in the formulation of designs germane to the oral cavity while tailoring the challenges of the physicochemical properties of the drug entity itself to achieve the drug delivery system's therapeutic aims. Strategically, these systems must address one or more of the following: 1. attain rapid release and absorption; 2. sustain the release and/or duration of the absorption process; 3. develop bidirectional or unidirectional systems; and 4. fabricate patient-friendly oral mucosal delivery systems. Indeed, patient compliance has recently shifted the trend towards the need for once a day dosing regimens and convenience. This has advocated the investigation of drugs with high potency and sustained effect (e.g. Peptide drugs). However, due to the advantages of delivering a drug through the oral mucosa, these drugs are viable candidates for delivery via this route.

1.9.1. Oral cavity

The Mucosa

When selecting drug candidates for incorporation into dosage forms for oral transmucosal drug delivery, one must be cognizant of the physicochemical properties of the drug molecule and their effect on absorption. However, the anatomy and physiology of the intended site for delivery also need to be recognized and evaluated.

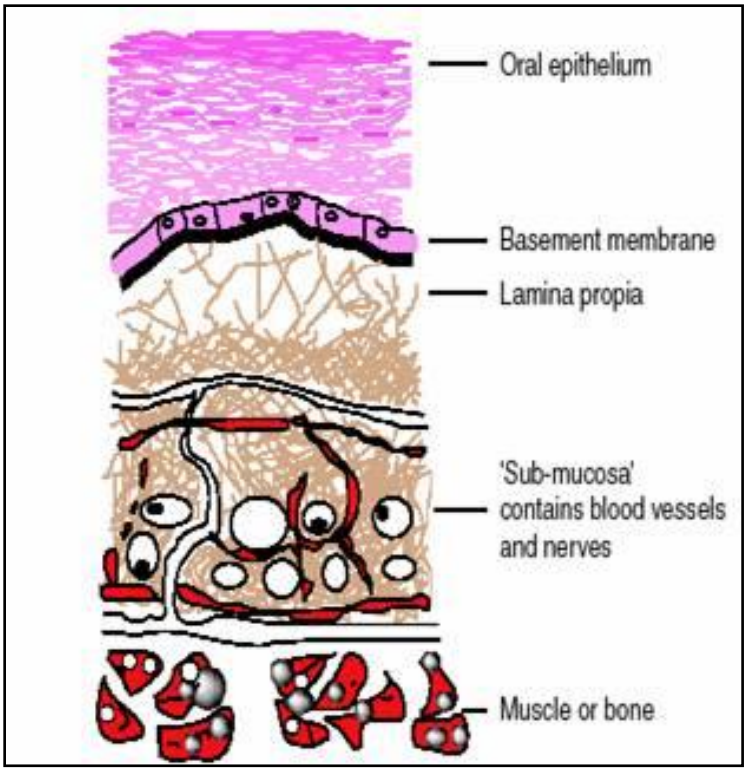

Figure 1-5: Structure of oral mucosa

General Mucosal Variation

All covering and lining tissues of the body consist of a surface epithelium supported by a fibrous connective tissue.[100] Upon comparing the structure of skin and oral mucosa to that of the GI tract, a major difference becomes apparent in the organization of the epithelium, which

reflects the different functions of these regions. The lining of the stomach, and the small and large intestine consist of a simple epithelium. However, skin and oral mucosa are covered by a stratified epithelium composed of multiple layers of cells, which show various patterns of differentiation between the deepest cell layer and the surface (Figure 1-5). This type of differentiation reflects the functional purposes and demands exerted on the tissue, such as mobility or rigidity and resistance to mechanical damage.

Regional Variation in the Oral Mucosa

In general, three different types of oral mucosa are recognized: 1. the masticatory mucosa; 2. specialized mucosa; and 3. the lining mucosa. Masticatory mucosa covers the gingiva and hard palate, which are regions that are subjected to the mechanical forces of mastication. This tissue consists of a keratinized epithelium that resembles the epidermis of the skin and is usually tightly attached to underlying structures by a collagenous connective tissue. Specialized mucosa, with characteristics of both masticatory and lining mucosa, is found on the dorsal of the tongue. It has a surface consisting of areas of both keratinized and non-keratinized epithelium, which is tightly bound to the underlying muscle of the tongue.

The lining mucosa of the oral cavity is covered by a stratified, squamous epithelium that is non-keratinized. This lining mucosa epithelium is located within the cheek (buccal), soft palate (palatal), part of the tongue (lingual), the floor of the mouth (sublingual), and the alveolar lining mucosa (labial mucosa). The buccal, labial and sublingual tissue are primary targets for drug delivery due to their potential permeability. Figure 1-5 illustrates these anatomical locations within the oral cavity as well as their mucosal type. Although the surface area of the oral mucosa is relatively small in comparison to the skin and the GI tract, its high vasculature

lends itself to potential drug access. As discussed previously, compounds that enter via the oral mucosa directly access the systemic circulation, thus avoiding first pass metabolism. Blood flow through the oral mucosa is abundant, and thus this factor is not considered a rate limiting step for drug absorption.

In a recent study comparing two types of oral mucosal absorption routes, Artusi et al[94] designed two thiocolchicoside dosage forms, one for sublingual and the other for buccal delivery. The buccal site demonstrated slower drug absorption than the sublingual route, however formulation bioadhesivity to the buccal site suggested that this was more suitable for sustained drug release. Thus, regional mucosa variation may be exploited for achieving oral transmucosal delivery objectives.

1.9.2. Advantages and challenges of the buccal drug delivery

The membranes that line the oral cavity are readily accessible, robust and exhibit fast cellular recovery following local stress or damage. Indeed, the oral cavity is very used to being exposed to various exogenous compounds. Properly constructed oral transmucosal drug delivery (TMD) systems are easy and painless to administer and well accepted by the patient. Precise dosage form localization is possible and there is the ability to terminate delivery when required (e.g. toxicity). Patients could conceivably control the period of administration, in addition to the desired therapeutic effect. From a formulation point of view, the thin mucin film that exists on the surface of the oral cavity provides the pharmaceutical scientist with the opportunity to retain delivery systems in contact with the mucosa for prolonged periods with the help of mucoadhesive compounds. Such a system would be in close contact with the absorbing membrane, thus optimizing the drug concentration gradient across the biological membrane and

reducing the diffusional pathway. Therefore, the oral transmucosal route is considered a potential route not only for rapid onset, but also for controlled or sustained systemic delivery.

For example, melatonin is a hormone secreted primarily by the pineal gland in a circadian fashion and acts as a synchronizer of biological rhythms in several animal species including humans. Because of the short half-life and poor oral bioavailability characteristics of melatonin, it is not a good candidate for conventional oral immediate-release delivery. A sustained- or controlled-release delivery system for melatonin with rapid onset and rapid offset may be necessary to mimic the normal endogenous plasma melatonin profile. Benes et al[101,102] evaluated the effect of oral controlled-release (CR), oral transmucosal (buccal, TMD) and transdermal (TDD) drug delivery systems on plasma concentrations of melatonin and its principal metabolite in human subjects. Inter-subject plasma concentrations of melatonin were significantly variable following both oral CR and TDD. TMD provided prompt systemic drug levels with less variability than oral CR or TDD delivery. Also, plasma melatonin levels fell promptly and rapidly after removal of the patch. These investigators concluded that the oral transmucosal route was able to mimic the physiological plasma profiles of both melatonin and its principal metabolite.

Disadvantages of transmucosal drug delivery entail an enzymatic barrier[103] and the physiology and histology of the various mucosa. The former leads to the degradation of peptides and proteins and the latter prevents the transport of large molecules. However, tailoring the formulation of oral transmucosal drug delivery systems to include enzyme inhibitors, when appropriate and permeation enhancers may minimize or eliminate these barriers. Thus, the numerous advantages of oral transmucosal delivery must continue to be explored for potential delivery of inorganic and organic agents for local as well as systemic therapeutic effects.

1.9.3. Factors Affecting Drug Absorption from the Oral Mucosa

Until the mid-1980s, only a handful of drugs were regarded to be feasible, or even clinically necessary, for delivery via the oral mucosal route. However, new and more potent drugs, and improved biomaterials have provided a renaissance for this route of delivery. In addition, with research in the transdermal area, it was demonstrated that certain penetration enhancers may be effective in improving the permeability of oral mucosal tissue, coupled with more appropriate delivery system designs (buccal patches, films). It therefore became necessary to understand the biological and physicochemical nature of the drug absorption process and the mechanism(s) of drug penetration across this tissue at the cellular and molecular level. The three main factors that influence drug absorption from the mouth entail the physicochemical properties of the drug, biological factors of the oral cavity and formulation factors of the drug delivery system.

Physicochemical Factors

Passive diffusion is the primary mechanism involved in drug permeation across the oral mucosa. Facilitated diffusion has also been shown to occur primarily with nutrients. The phenomenon of "buccal partitioning" has been used to describe the membrane reservoir effect noted in some studies.[104-107] However, this drug binding effect has not been well identified to date. Parameters such as partition coefficient (K_p), degree of ionization and molecular weight of the drug itself are of paramount importance for drug delivery across the oral mucosa membrane. The extent of oral mucosal absorption is generally proportional to the drug's lipophilicity or oil-in-water partition coefficient. However, there is a delicate balance between K_p and solubility for a drug to be a successful candidate for oral mucosal absorption.[108] For example, if a drug is too

lipophilic, it cannot dissolve appreciably in the aqueous mucin/saliva and thus may not be available for significant absorption. Therefore, potency of the drug must be considered when choosing an appropriate drug candidate for a transmucosal delivery system.

Generally, the unionized form of a drug is considered to be the most lipid-soluble and thus most diffusible across biological membranes. Therefore, pK_a of the drug must be addressed when diffusivity and ultimate bioavailability for a therapeutic agent are being considered. The average pH of human saliva is approximately 6.4 and the importance of pH on drug absorption via the oral mucosa has been studied extensively. Although most of these studies illustrate the importance of the state of drug ionization, the pH of saliva remains relatively constant. Thus the pK_a of a weak acid or a weak base ultimately is the deciding factor as to the degree of drug ionization. In general, small molecules penetrate the mucosa more rapidly than larger molecules. However, concerning molecular weight, it should be noted that inclusion of penetration enhancers has dramatically improved the permeability of some high molecular weight mucopolysaccarides.[109]

Biological Factors

Saliva may be considered a positive or a negative factor for oral transmucosal drug absorption. It has been reported that adult males have an average volume of approximately 0.9 mL and females a slightly smaller average volume of about 0.8 mL.[109,110] Studies of salivary secretions have established that mucins together with other groups of salivary proteins such as proline-rich proteins, histatins, cystatins, statherins and α-amylases protect the integrity of the hard and soft oral tissues. Changes in both the volume and composition could alter the permeability of the mucosa. The salivary 'component' is important in that drug absorption

occurs more readily across moist mucous membranes than those that lack mucous.[111] Also, a drug must dissolve before absorption can occur—and in TMD systems the dissolution medium is aqueous mucin/saliva. But excess saliva has been shown to adversely affect absorption for some systems. On the other hand, disease states or other drugs that the patient may be taking may cause xerostomia and again may inhibit drug permeation due to decreased salivary flow. These factors may in turn affect the condition of the mucosa, which may cause an increase or decrease in drug penetration.

A limiting factor for oral mucosal drug delivery is the area to which the delivery system may be applied in the oral cavity.

It is generally agreed that the oral mucosa tissue is more permeable to drugs than the skin. The extent of differences in permeability, however, is widely debated. These estimates in the literature range from a 4-fold to a 4000-fold greater permeability for the mucosa.[112] Such wide differences partially lie in the fact that different regions of the mucosa exhibit different structures and thus different permeability. It is also generally accepted that the permeability 'barrier' exists in the outermost one-fourth to one-third of the mucosal epithelium. This barrier has been proposed to result from intercellular material that is derived from membrane coating granules (MCGs).[96] In keratinized epithelia these intercellular lipid substances include sphingomyelin, glucosylceramides, ceramides and other non-polar lipids. Unkeratinized epithelia contain primarily cholesterol esters, cholesterol and glycosphingolipids. Studies have demonstrated that the MCGs resulting decrease in permeability and keratinization alone may not play a significant role in the permeability barrier. Due to other factors discussed previously, a primary strategy for transmucosal drug delivery should be to target the oral cavity's lining mucosa.[113] Oral mucosal anatomy suggests that there are two permeability barriers. The

intercellular spaces and cytoplasm are essentially hydrophilic in nature and function as a transport barrier for lipophilic compounds. The second barrier is the cell membrane, which is lipophilic and impedes diffusion of hydrophilic compounds due to a low partition coefficient. The coexistence of these two regions in the oral mucosa suggests two possible routes for drug transport: paracellular (intercellular) and transcellular (intracellular). Drug transport by these two routes most likely occurs simultaneously, although one route usually dominates depending on the physicochemical properties of the diffusant. It is believed by some researchers that the paracellular route is the primary route for hydrophilic compounds, and thus, the intercellular space is the preferred route for hydrophilic drug transport. Limited surface area of the intercellular space and the labyrinthal pathways within this area are the main limitations for this route. The flux of a drug candidate in this route (J_H) may be written as:[114]

$$J_H = D_H \varepsilon C_D / h_H \qquad (4)$$

where ε is the fraction of surface area of the paracellular route, D_H is the diffusion coefficient of the intercellular spaces, h_H is the path length of the paracellular route and C_D is the donor side drug concentration.

The cell membrane is relatively lipophilic in nature and thus for lipophilic compounds the partition coefficients are high. Also, the surface area for the transcellular route is large and the pathways for transcellular movement is relatively short, thus it is believed that the permeability of lipophilic compounds across the epithelial cell membrane is typically high. The flux of drug movement in the transcellular route (J_L) can be expressed as:

$$J_L = (1-\varepsilon) D_L K_P C_D / h_L \qquad (5)$$

where K_P is the partition coefficient between the lipophilic region (cell membrane) and the hydrophilic region, h_L is the path length of the transcellular route. Regardless, one should keep in mind that the primary route of transport is that which provides the least resistance to penetration of an individual drug molecule.

1.9.4. Requirements for a Transmucosal Drug Delivery System—Formulation Factors

Bioadhesion and Bioadhesive Polymers

Bioadhesion is a phenomenon related to the ability of biological or synthetic material to adhere to a biological substrate. Oral mucosal drug delivery necessitates the use of mucoadhesive polymers as these dosage forms should ideally adhere to the mucosa and withstand salivation, tongue movement and swallowing for a predetermined period of time. As one would expect, this concept has received considerable attention in the pharmaceutical field due to the potential for applications in drug delivery. A widely used approach to explain the adhesive properties of drug delivery systems is based on the belief that interatomic or inter-molecular forces are established at the interface of the adhesive and the substrate. Numerous mechanisms of adhesion or mucoadhesion have been established and proposed. These include hydrogen bonding, surface energy and contact angle considerations, polymer chain interpenetration, and the swelling rate of a polymer interacting with mucin.[115-117] Adequate bioadhesion is essential for the success of a bioadhesive drug delivery system such that controlled release is attained to elicit the desired therapeutic response. Examples of mucoadhesive polymers that have been investigated are Carbopol, POLYOX™ etc.

Several techniques for in-vitro determination of bioadhesion have been reported, which include tensile testing, shear stress testing, adhesion weight method, fluorescent probe method, flow channel techniques and a colloidal gold staining method. More recently, Texture Analyzer® (TA.XT2i) has been used for mucoadhesive studies. Some of the texture analyzer variables that may be used to assess a transmucosal system's bioadhesion are contact force, contact time and speed of probe withdrawal from the chosen substrate.[118,119]

Penetration Enhancement of the Oral Mucosa

Though the oral cavity has been used as a site for systemic drug delivery, the amount of drug absorbed via this route has historically been problematic for the attainment of a desired therapeutic response. As discussed previously, this is due in part to the relatively small surface area of the oral mucosa and, more importantly, relatively low tissue permeability to various therapeutic agents. Unlike surface area, which is relatively constant, permeability of the oral mucosa may be temporarily altered to allow a greater drug flux and thus increased bioavailability.[120,121] Such an increase in drug permeability via the oral epithelium has been demonstrated in several studies by the use of chemical penetration enhancers.[121-125]

An ideal chemical penetration enhancer would possess the following properties: 1. Non-toxic, non-irritating, non-allergenic; 2. Immediate onset of increased permeability; 3. Immediate recovery of the normal barrier properties when removed; and 4. Physically and chemically compatible with a wide range of drugs. It is not surprising that such an ideal enhancer has not been discovered to date. An improved understanding of enhancer structure and mechanism of action is extremely important for formulation choices of transmucosal systems. In general, penetration enhancers are thought to function in one or more of the following manners:

Fluidizing intercellular lipids, altering protein conformation and/or modifying drug solubility parameters.[123,124] In a formulation, judicious use of a combination of enhancers may provide a synergistic impact on membrane permeability while minimizing individual enhancer concentration and thus decreasing tissue toxicity (irreversibility). A study of current penetration agents coupled with the discovery of novel agents will lead to a new era in oral mucosal drug delivery systems. Also a continued and intensified effort is needed to thoroughly explore the enhancer agents currently available.

Enzyme Inhibitors

A majority of the protein and peptide degradation events that occur in the GI tract revolve around the resident proteases and peptidases (e.g. pepsin, chymotrypsin, elastase, carboxypeptidase, aminopeptidase).[126,127] Although buccal mucosa is relatively low in enzymatic activity as compared to other mucosa, there is still potential for degradation of drugs delivered via the intra-oral transmucosal route. This transformation is particularly true for peptide/protein drugs due to the presence of enzymes in the saliva and mucosa. Therefore, this enzymatic activity may act as a barrier to drug transport that limits the absorption of drugs across the tissue. Walker et al.[126] evaluated the peptidase activity on the surface of porcine buccal mucosa in vitro and reported that, while aminopeptidase N activity was detected using Leu-*p*-nitroanilide, no carboxypeptidase or dipeptidyl peptidase IV activity was detected on the buccal mucosa. In such cases, enzyme activity can be minimized by the co-administration of enzyme inhibitors (e.g. bacitracin, aprotinin, diprotin-A) to improve the bioavailability of drugs via buccal delivery. Langoth[127] , Yang[128,129] , Bird[130] and Garren[131] have demonstrated the role of enzyme inhibitors in their respective studies

1.9.5. Oral Transmucosal Dosage Forms

Conventional Dosage Forms for Oral Transmucosal Delivery

Solutions and various types of tablets, including fast dissolving tablets, are conventional dosage forms that have been adapted for oral transmucosal use. The primary focus of these delivery systems is to rapidly create high drug concentrations within the mouth and make the therapeutic agent available to a relatively large mucosal surface. These dosage forms are generally easy to manufacture since standard manufacturing techniques may be utilized. However, several formulation and physiological factors may make their performance as oral mucosal delivery systems less than optimal for both rapid and sustained drug delivery. These factors may include: 1. short residence time of such formulations as they may be removed by swallowing; 2. high inter- and intra-individual variability in bioavailability; and 3. the released drug is not protected from the environment and therefore subject to its influences. In addition, following administration of these dosage forms, the drug concentration falls rapidly due to extensive dilution by saliva and the resultant swallowing of the drug by the patient. Therefore, the formulations that balance the rate of drug release with the rate of drug absorption for the attainment of controlled or pre-determined drug delivery are necessary. The system must be of a surface area and thickness acceptable to the patient while containing and releasing sufficient drug for the desired therapeutic activity. It has been reported that intra-oral patches having a surface area of 0.5 to 1 cm^2 are comfortable and patient-friendly, although larger patches may be tolerated. Convenient, patient friendly transmucosal thin films can be prepared by various methods such as solvent casting and hot-melt extrusion. Films prepared by hot-melt extrusion have definite advantages of being pliable and strong.

1.10. Hot-Melt Extrusion:

Hot-melt extrusion (HME) is the process of pumping raw materials with a rotating screw under elevated temperature through a die into a product of uniform shape. Although used for over half a century in the plastics industry, interest in HME techniques for pharmaceutical applications is growing rapidly with well over 100 papers published in the scientific literature in the last 12 years. Also, the number of HME patents issued for pharmaceutical systems has steadily increased since the early 1980's with international scope (Figure 1-6).[132] Within the chart below, the US and Germany hold approximately 28% of the patents each, while Japan makes up about 19% of the total.

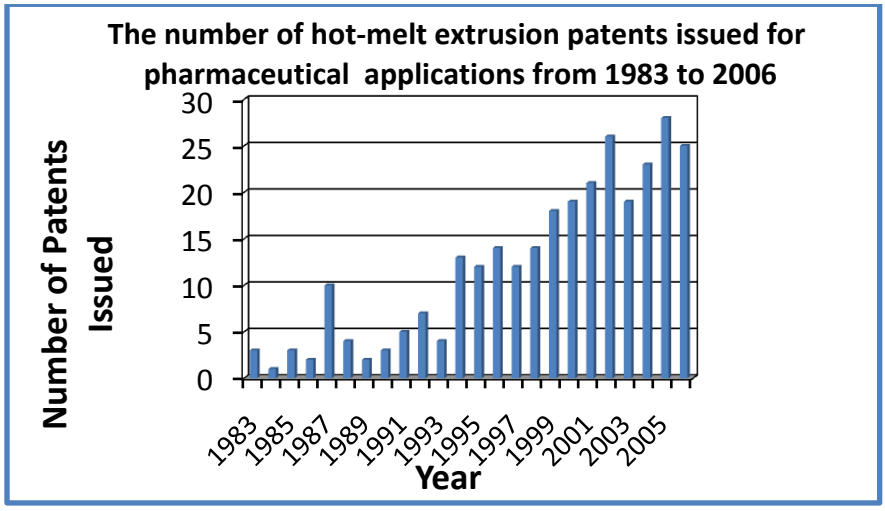

Figure 1-6: Patents for hot-melt extrusion

HME offers many advantages over other pharmaceutical processing techniques. Molten polymers during the extrusion process can function as thermal binders and act as drug depots and/or drug release retardants upon cooling and solidification. Solvents and water are not necessary thereby reducing the number of processing steps and eliminating time-consuming drying steps. A matrix can be massed into a larger unit independent of compression properties.

The intense mixing and agitation imposed by the rotating screw cause de-aggregation of suspended particles in the molten polymer resulting in a more uniform dispersion. The process is thus continuous and efficient.

It has been estimated that as many as 40% of all new molecular entities have poor bioavailability because of low aqueous solubility. This percentage is likely increasing due to the advent of combinatorial chemistry and the importance of lipophilic receptors. Formulation of such compounds for oral delivery presents one of the most frequent and formidable challenges to formulation scientists. HME has been used to improve the bioavailability of drug substances especially those having low water solubility by formation of molecular dispersions.[133-137]

HME requires a pharmaceutical grade polymer that can be processed at relatively low temperatures due to the thermal sensitivity of many drugs. All components must be thermally stable at the processing temperature during the short duration of the heating process. Although this requirement may sometimes limit a pharmaceutical compound from HME processing, input of new techniques and equipment specifications over the last decade have expanded the list of actives not previously thought to be applicable for this emerging technology. Table 1-1 summarizes the advantages and disadvantages of HME.

Table. 1-1: Advantages and Disadvantages of Hot-Melt Extrusion Processing

Advantages	Disadvantages
✓ HME is a potential "continuous process"	✓ May not be applicable for heat labile drugs (such as proteins, peptides, etc.)
✓ No organic solvents or water are needed	✓ Must be relatively moisture free
✓ Less labor and equipment demands	✓ Non-traditional equipment and requires education and training
✓ Shorter and more efficient processing times	
✓ Favorable product cost	
✓ Can produce "solid solutions or dispersions" which may lead to improved solubility and bioavailability	
✓ Product life-cycle management	

1.10.1. Equipment, Principles of Extrusion and Process Technology

***HME Equipment*:** Pharmaceutical-class extruders have evolved and adapted to mix drugs with carriers for various solid dosage forms as well as for the production of wet granulations. The major differences between a plastics extruder and a pharmaceutical-class extruder are the contact parts, which must meet regulatory requirements. Typically, the metallurgy of the contact parts must not to be reactive, additive or absorptive with the product. In addition, the equipment is configured for the cleaning and validation requirements associated with a pharmaceutical environment. For example, some pharma extruders offer a 'clam shell' design for ease of access to the screw. Also, HME research at the lab scale has lead to the development of 'mini' extruders so that feasibility studies may be performed on small quantities of polymers and APIs. Otherwise, the unit operations performed for a pharmaceutical product is virtually identical to a plastics extrusion process.

Types of Extruders and Screw Design: Most pharmaceutical extrusion applications utilize 'screw extrusion' rather than ram extrusion due to better control of temperature profiles and product homogeneity. Thus unlike ram extrusion, a screw extruder provides more shear stress and intense mixing. At a minimum, a screw extruder consists of three distinct parts: a conveying system for material transport and mixing, a die system for extrudate formation and downstream auxiliary equipment for cooling, cutting or collecting the extruded product. Individual components within the extruder are the feed hopper, a temperature controlled barrel, a rotating screw, die and heating and cooling systems. Standard process control and monitoring devices include zone temperature and screw speed with optional monitoring of torque, drive amperage, pressure and melt viscosity. Temperatures are normally controlled by electrical heating bands, and monitored by thermocouples. Basically, there are two types of screw extruders: 1) Single screw and 2) Twin screw

The single screw extruder is the most widely used extrusion system in the world (especially for plastics). One screw rotates inside the barrel and is used for feeding, melting, devolatilizing and pumping. However, once screws are reduced to less than 18 mm, the screw becomes weak and solids transportation is far less reliable. To overcome these shortcomings, a vertical screw, driven from the discharge end, may be used.

Twin-screw extruders, employed increasingly in pharma applications, utilize two screws usually arranged side by side. The use of two screws allows a number of different configurations to be obtained and imposes different conditions on all zones of the extruder, from the transfer of material from the hopper to the screw, all the way to the metered pumping zone.[138] In a twin-screw extruder, the screws can either rotate in the same (co-rotating extruder) or the opposite (counter-rotating extruder) direction. Generally, counter-rotating twin-screw extruders suffer

from disadvantages of potential air entrapment, high-pressure generation, and low maximum screw speeds and output, and are thus not used routinely for pharma applications. Co-rotating twin-screw extruders on the other hand are generally of the intermeshing design, and are thus self-wiping.[139] They are industrially the most important type of extruders and can be operated at high screw speeds and achieve high outputs, while maintaining good mixing and conveying characteristics. Twin-screw extruders have several advantages over single screw extruders, such as easier material feeding, high kneading and dispersing capacities, less tendency to over-heat and shorter transit time.

In an extrusion process, the dimensions of the screws are given in terms of L/D ratio, which is the length of the screw divided by the diameter. Typical extrusion process lengths are in the 20 to 40:1 L/D range, or longer. Extruder residence times are generally between 5 seconds and 10 minutes, depending upon the L/D ratio, type of extruder, screw design and how it is operated. The size of an extruder is generally described based on the diameter of the screw used in the system, i.e., 16 – 27 mm extruder (pilot scale) as compared with a 60 mm extruder (production scale).[140] The screw is typically divided into three sections along the length of the barrel: feeding, melting or compression, and metering. The die is attached at the end of the barrel. The shape of the die dictates the physical form or shape of the extrudate. A wide variety of downstream systems are available following the extrusion process. Pellets or shapes may be extruded and wound or cut-to-length. Co-extrusion allows the possibility of complex properties from a single structure, which can be beneficial for time-release products. Film and lamination systems are used to combine melt extrusion with substrates for transmucosal and transdermal applications. For film applications, chill rolls and torque winders are used to rapidly cool and collect the extrudate. Film thickness can be adjusted by changing the die opening, the mass flow

rate introduced into the extruder, screw speed, the rotation speed of the chill rolls, or the torque winder.

The efficiency of the melting process depends on the polymer properties and the extruder design. Thermoplastic polymers primarily exist in a molten state when entering the metering section. The mass flow rate of the extrudate is highly dependent upon the channel depth and the length of the metering section. In general, polymers with low melt viscosities and high thermal conductivities exhibit a more efficient melting process. Changes in the screw design are sometimes warranted to improve the melting process and improve mass flow through the extruder. Solidified polymer components can block the channel if melting is incomplete and result in a surge of material around the blockage. Differential scanning calorimetry, thermogravimetric analysis and gel permeation chromatography are often used to monitor polymer stability. Plasticizers, antioxidants, thermal lubricants and other additives are often included in the formulation to address stability issues.

1.10.2. Applications of HME

In recent years, several research groups have demonstrated HME as an innovative and viable approach to produce various pharmaceutical drug delivery systems in the field of formulation such as pellets[141,142], granules[143-145], immediate and modified release tablets[146,147], oral fast dissolving systems[148], transdermal and transmucosal delivery systems[149-151], transungual delivery systems.[152]

HME technology has proved its potential in producing various solid dosage forms, providing the flexibility to modify drug release as required. This part of the article will focus on various HME applications in drug delivery through oral, transdermal, transmucosal, transungual, and other routes of administration.

Oral Drug delivery: A United States patent, McGinity et al.[145,153,154] has disclosed a novel method of preparing effervescent granules utilizing hot-melt extrusion techniques. The granules were prepared by hot-melt extruding an acidic and an alkaline agent coupled with a hot-melt extrudable binder (melting/softening point temperature less than 150°C), which was capable of forming a eutectic mixture with the acidic agent. The granules thus produced demonstrated a controllable rate of effervescence. Additionally, hot-melt extrusion has been utilized to enhance the dissolution rate of the actives by preparing solid dispersions for immediate and sustained release applications. Sun Yunzhe et al. prepared a semi-solid capsule containing nimodipine solid dispersion prepared by HME technology. The bioavailability study of this HME formulation in the capsule conducted in beagle dogs exhibited similar bioavailability to a referenced product. However, time to reach peak concentration was much faster for the HME product than the reference formulation. In this study authors have demonstrated that the combination of a solid dispersion technique and semi-solid filling into the capsule not only produced a rapid and pH-independent release of the drug, but also prevented recrystallization of the drug in the matrix.[155] The above examples illustrate the fact that the choice of excipients is of utmost importance in designing HME dosage forms with fast or immediate release characteristics.

Controlled Release **:** Fukuda, Peppas and McGinity investigated the influence of sodium bicarbonate on the physicochemical properties of controlled release HME tablets containing ammonio methacrylate copolymer, Type B (MAC RS PO) and/or amino methacrylate copolymer (MAC E PO).[156] In this study, acetohydroxamic acid and chlorpheniramine maleate were used as the model drugs. The authors studied the drug release properties and buoyancy in the dissolution media for both HME and directly compressed (DC) tablets by incorporating sodium bicarbonate

into the tablet. The HME tablets prepared from the powder blend containing both MAC RS PO and sodium bicarbonate demonstrated sustained release properties. In addition, the tablets floated on the surface of the dissolution media for up to 24h. The cross-sectional morphology of the hot-melt extruded tablets exhibited a porous structure, reportedly due to carbon dioxide gas generation resulting from thermal decomposition of sodium bicarbonate in the softened acrylic polymers at elevated temperature during processing. On the contrary, the DC tablets exhibited rapid drug release in the dissolution media and did not demonstrate any buoyancy. The drug release rate from floating HME tablets was controlled by both the incorporation of MAC E PO into the matrix tablet and the diameter of the die utilized in the extrusion equipment.

1.10.3. HME and Advanced Technologies

In the recent decade, drug particle engineering, utilizing nanotechnology, has gained much interest to improve the solubility limitations of poorly soluble drug compounds. However, it is associated with several limitations such as particle aggregation, morphological instability, and poor wettability. Miller and co-workers demonstrated the suitability of HME as a novel and viable approach for nanoparticle engineering by overcoming the above mentioned limitations.[157] The focus of this investigation was to elucidate whether the shear generated during extrusion would deaggregate and disperse the micronized particles into a hydrophilic polymer matrix, without solubilizing or altering the properties of the drug particles. The concept was evaluated by developing itraconazole (ITZ)-polyvinyl pyrrolidone (PVP) and ITZ-hydroxypropyl methyl cellulose (HPMC) microparticles, subsequently dispersed in a polymer carrier system composed of poloxamer 407 and polyethylene oxide (PEO)-200M (7:3 ratio) utilizing a melt extrusion technique. From SEM imaging, authors have provided visual confirmation and demonstrated that HME did not alter the morphology of the engineered particles and that they were homogeneously

dispersed within the polymer carrier system. Moreover, drug release studies performed revealed that the dissolution rate of the micronized particles was improved by HME due to particle deaggregation and enhanced wetting. However, the selection of a carrier system and optimization of operating conditions during the extrusion process were considered the critical steps utilizing this methodology. It was determined that the two extruded formulations performed similarly in their *in vivo* rat model, per oral dosing, as confirmed by their statistically similar AUC values

1.10.4. HME and Transmucosal Drug Delivery

Transmucosal delivery is particularly advantageous for poorly-water soluble drugs and those which undergo extensive first-pass metabolism. Repka and co-workers extensively investigated the hot-melt extrusion technique as an incisive means for developing numerous formulations containing various drug molecules for delivery through the buccal route.[152,158-161]

Polymeric films intended for oral or transmucosal delivery should be flexible, elastic and soft, yet sufficiently bioadhesive to withstand the mechanical stress of the oral cavity. Prodduturi et al. developed clotrimazole (CT) polymeric films utilizing different molecular weights of hydroxypropyl cellulose (HPC- JF, GF and MF) and polyethylene oxide (PEO N-80 and PEO N-750).[162] Both the polymeric systems exhibited zero order drug release and release rate was dependent on the molecular weight of the polymer. The drug release rate constant and release mechanism were independent of % of drug loading. Thus, size of the dosage form and/or release of the drug from extruded film could be tailored by altering the drug load without affecting release mechanism. Also, it was determined that at a minimum of a 55:35 ratio of polymers (HPC/PEO) produced optimal long term stability of the API. These data are obviously germane for the development of successful controlled release dosage forms. Influence of physico-mechanical properties of vitamin E TPGS, an amphiphilic molecule, as a formulation additive on

the properties of hydrophilic films was studied by Repka and McGinity.[160] Vitamin E TPGS functioned as a plasticizer since a linear decrease in glass transition temperature of the films, containing either a 50:50 or 80:20 ratio of HPC to PEO, with increasing concentrations of Vitamin E TPGS (1, 3, and 5%) was observed. The reported effect was comparable to other conventional plasticizers. In addition, vitamin E TPGS was found to be an excellent processing aid, by decreasing barrel pressure, drive amps, and torque of the extruder.

1.11. Conclusion

Hot-melt extrusion technology is an increasingly attractive process for the manufacture of various drug delivery systems. The literature published in this field so far reveals many interesting aspects such as fast dispersing systems, floating systems, complex formation within the melt, and nanoparticle engineering combined with melt extrusion technology. Many of the pharmaceutical products prepared utilizing this technique has been approved in the United States, Europe, and Asia. The drug being embedded in the carrier matrices as a solid dispersion/solution may allow for sustained or controlled release applications and dissolution rate improvement. Moreover, improved bioavailability has been achieved which demonstrates the value of HME as a powerful drug delivery technology. Although several researchers have been successful in employing HME to formulate many thermolabile molecules, exploitation of innovative pharma equipment design, new custom designed polymers and novel processing approaches continues for the development of even more efficient drug delivery systems. Due to the advantages offered by HME, it will be employed as a primary technology to produce hot-melt polymeric transmucosal films of THC-HS.

CHAPTER 2

Goals and Objectives

With the potential problems associated with transmucosal delivery of THC-HS in mind, the main objectives of this research are:

***Chapter 3**: To develop novel solid dispersions of THC-HS with cyclodextrins as solubilizing agents*

Preparation and characterization of cyclodextrin complexes of THC-HS by different techniques:

- ✓ Phase solubility
- ✓ NMR spectroscopy
- ✓ UV spectroscopy
- ✓ FT-IR spectroscopy
- ✓ Molecular Modeling

***Chapter 4**: Assessment of chemical and enzymatic stability of THC-HS:cyclodextrin complexes in solution-state*

Chemical stability and enzymatic hydrolysis studies in buccal homogenates were performed:

- ✓ pH degradation profile
- ✓ Enzymatic hydrolysis studies in buccal homogenates
- ✓ *In vitro* buccal permeability of aqueous solutions of THC-HS:cyclodextrin complexes

***Chapter 5**: Preparation of solid dispersions of THC-HS:cyclodextrins and study of their thermal stability and release characteristics*

- ✓ Accelerated stability testing of solid dispersions of THC-HS:cyclodextrins

- ✓ Dissolution release profile characterization of solid dispersions of THC-HS:cyclodextrin complexes
- ✓ In vitro buccal permeability of solid dispersions of THC-HS:cyclodextrins

Chapter 6*: Formulation studies of hot-melt cast films of THC-HS-RAMEB solid dispersions*

- ✓ Effect of processing temperature on stability of THC-HS:RAMEB complex in transmucosal films
- ✓ Content uniformity of hot-melt cast films containing THC-HS:RAMEB complex in transmucosal films
- ✓ Effect of PEO-N80 on the stability of THC-HS:cyclodextrin solid dispersions in polymeric transmucosal matrix system
- ✓ Effect of plasticizers/processing aids on the stability of THC-HS:cyclodextrin solid dispersions in polymeric transmucosal matrix system
- ✓ Effect of various pH modulators to modulate the microenvironmental pH and stabilize the prodrug in polymeric films
- ✓ Effect of anti-oxidants and their combinations on stability of THC-HS incorporated into the matrix patch system

Chapter 7: *Design of Experiments approach to identify critical formulation variables*

Chapter 8*: Preformulation studies of* Δ^9*-Tetrahydrocannabinol aminophenyl butyrate (THC-APB)*

- ✓ To study the pH solubility and pH stability profile of THC-APB
- ✓ To study the feasibility of incorporating THC-APB in hot-melt films

CHAPTER 3

Preparation and characterization of inclusion complexes of a hemisuccinate ester prodrug of Δ^9-tetrahydrocannabinol with modified beta-cyclodextrins

3.1. Introduction

Δ^9-Tetrahydrocannabinol is the primary active ingredient of the plant *Cannabis sativa* (marijuana) and is responsible for the majority of the pharmacological effects.[163] While THC in marijuana is mainly known for its abuse potential, it also exhibits therapeutic effects in the treatment of nausea and vomiting during cancer chemotherapy, in appetite stimulation, cachexia associated with AIDS, glaucoma, analgesia, and other indications.[164-168] To date, the most promising clinical applications approved by the Food and Drug Administration (FDA) are for the control of nausea and vomiting associated with chemotherapy and for appetite stimulation of AIDS patients suffering from anorexia and wasting syndrome. It is significant to note that the only dosage form currently approved by FDA is an oral, soft gelatin capsule (e.g. Marinol®). In addition, orally administered THC has shown slow and variable absorption due to low oral solubility/permeability and first pass metabolism.[168] The alternative routes of administration such as inhalation/smoking of THC have demonstrated more consistent and higher plasma drug levels; however, these methods are not desirable as they bear the potential of drug abuse. Moreover, the efficacy of smoked THC is largely dependent on the experience level of the subject with experienced smokers being able to self-titrate to achieve the desired symptomatic relief. Parenteral drug delivery of THC is besieged with the problems of being invasive and requiring professional assistance, and therefore in many cases precludes self medication. In addition, this route is inherently subject to abuse. These limitations of the alternative routes of administration underline the need to explore various drug delivery strategies for oral administration of THC. The recent advances in drug delivery technologies make an oral administration an attractive approach to redevelop THC formulations without its earlier limitations. The problems associated with low solubility/permeability and first-pass metabolism can be overcome with the help of

currently developing supersaturating drug delivery systems. A variety of formulation approaches under the consortium of supersaturating delivery systems for intestinal absorption are being applied to solubilize and maintain the supersaturation of water-insoluble drugs for a time period sufficient for absorption.[169] They include solubilized formulations such as cosolvent systems, lipid-based formulations as well as high energy solids like amorphous, crystalline salt forms, co-crystals and prodrugs. Our strategy towards developing THC oral formulations uses a two-pronged approach a) development of a new generation of hydrophilic prodrugs of THC (use of high energy solids), and b) solubility enhancement and stabilization of prodrugs in oral formulations. The approach of synthesizing more hydrophilic prodrugs of the molecule has been successfully used in modifying the physico-chemical properties of the parent drug without changing the core structure of the compound.[170,171] The prodrugs thus synthesized cleave the parent drug at appropriate pH and protect the parent moiety from any chemical modification before it reaches its desired target.

Thus, a first generation prodrug of THC, the hemisuccinate ester of THC (THC-HS, Fig. 3-1) was synthesized. It is a light-yellow, viscous liquid that is sticky at room temperature and hardens upon refrigeration, suggesting that the glass transition temperature is below 25°C, thereby necessitating its storage under freezing conditions for stability purposes.

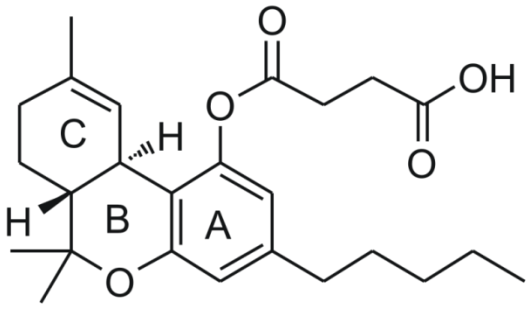

Figure 3-1: Structure of THC-HS

THC-HS has a molecular weight of 414.53 and logP of 3.33 (Moriguchi's method), is sparingly soluble in water, and mostly acidic with a pKa of 4.25 (ACD/Labs, Toronto, Ontario, Canada).[30,31] Indeed the prodrug itself is not without problems; one of the main issues being its high hydrolytic potential.

The other problems associated with transmucosal oral delivery of THC-HS are: a) high instability to heat and hydrolysis and prone to oxidation, b) the poor solubility of the prodrug (better than the parent drug) making its absorption, dissolution rate- limited, and c) since the prodrug is sticky and resinous in nature, handling issues are a major concern in formulation of the drug. Hence, suitable methods need to be employed to make it free-flowing. At this stage, a solubilization approach was adopted to enhance the solubility of THC-HS and maintain a higher concentration in the gastrointestinal tract (GIT) in order to enhance absorption. Earlier, attempts have been made by Repka et al to enhance the solubility and stability of THC-HS using different polymers, solubilizers, anti-oxidants and pH modifiers.[30,31,172,173] These solubilization methods were only moderately successful in increasing the solubility of THC-HS warranting a better solubilization strategy. One of the most commonly used technologies to enhance the solubility and, in turn, the absorption of water-insoluble drug molecules from the GIT is the use of cyclodextrin complexation of drugs.

Cyclodextrins (CDs) are cyclic oligosaccharides composed of 6-8 dextrose units (α-, β- and γ CDs respectively) joined through 1-4 bonds. Because the interior of these molecules are relatively hydrophobic and the exterior relatively hydrophilic, they tend to form inclusion complexes of the type shown in the Fig 3-2.

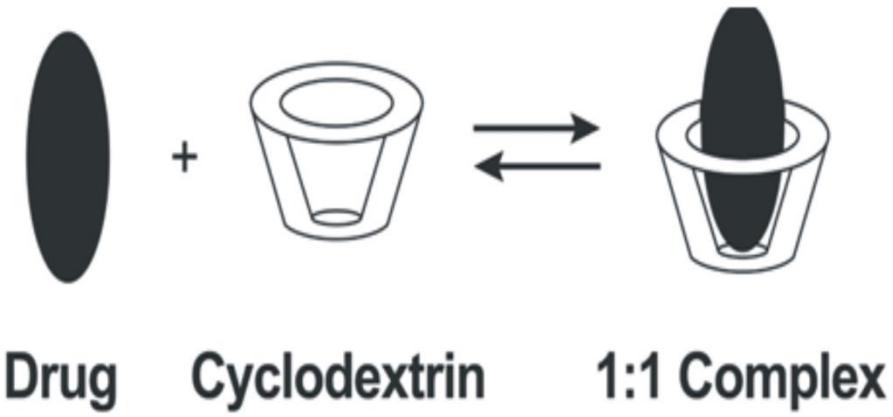

Figure 3-2: Equilibrium binding of a drug with a CD to form a 1:1 inclusion complex

α- and β-CDs are known to be parenterally unsafe due to severe nephrotoxicity caused by either CD uptake by the kidney tubule cells followed by disruption of intracellular function, or the extraction of lipid membrane components by the CD. Modification of the parent CDs to improve the safety, while maintaining the ability to form inclusion complexes with various substrates, has led to the synthesis of highly water-soluble random methylated beta-CD (RAMEB), 2-hydroxyprpoyl- beta-CD (HPBCD), sulfobutyl ether- beta-CD (SBECD), etc. These synthetically modified CDs have better solubility and excellent safety profiles compared to the parent CDs. They have been used in parenteral, oral, transdermal and ocular formulations. Synthetic CDs like RAMEB, HPBCD and SBECD are approved by regulatory agencies in the USA and Europe.[174-179]

By the virtue of being able to form inclusion complexes with the drugs, CDs are known to enhance the solubility and protect the ester linkage of the compounds from hydrolysis. A vast amount of literature is published supporting the enhancement of bioavailability for oral formulations due to the inclusion complex formation with CDs.[34,36,180-185] However, in very few cases the absorption was hindered due to CD binding to the free drug and thus reducing its free concentration.[184] In the process of inclusion complexation, the drug and CD are in dynamic

equilibrium with each other leading to the inhibition of precipitation upon dilution up to a certain threshold. This phenomenon leads to the formation of supersaturated solutions of drug and CDs in the GIT with an enhanced absorption profile. In addition, the drugs can be lyophilized with the modified CDs to yield free-flowing powders. As a result, it was determined worthwhile to explore these advantages offered by CDs to develop an oral formulation for this ester prodrug of THC (THC-HS). The range of effects produced by CD complexation on absorption of water-insoluble molecules is variable due to the chemical nature of the drugs, dose and drug: CD ratio. Hence it is difficult to predict *a priori* or create a generalized model of efficacy of CD with every drug molecule. Yet, it is important to understand the physical and chemical interactions of CDs with the individual drug molecule to predict the efficacy of the complexation process on that particular drug. As a first step towards the development of oral controlled formulations of THC-HS, we are reporting the preformulation studies of CD inclusion complexes with THC-HS. The objective of this work is to determine the feasibility of inclusion complex formation of THC-HS with two beta-CD derivatives to enhance the solubility of THC-HS. For this purpose, solid dispersions of THC-HS-beta -CD derivatives were prepared by different techniques (Coevaporation, adsorption and lyophilization) and characterized by proton NMR, Fourier transform Infrared spectroscopy (FT-IR), Job's plot and molecular modeling.

3.2. Materials

The following chemicals were used as received: randomly methylated beta-CD (RAMEB) and 2-hydroxyprpoyl- beta-CD (HPBCD) were purchased from Sigma Aldrich with a degree of substitution of 1.7 and 0.6, respectively. HPLC-grade water was freshly prepared in the laboratory (by Nanopure systems, Barnstead, Dubuque, IA). HPLC-grade acetonitrile and

methanol and were obtained from Fisher Scientific, Fair Lawn, NJ; THC-HS (in hexane) and THC (in absolute ethanol) were provided by ElSohly Laboratories Inc, Oxford, MS.

3.3. Methods

For preparation of the complexes, aqueous solutions of RAMEB/HPBCD and THC-HS were equilibrated for 24 h at 25°C under constant shaking at 100rpm. The samples containing THC-HS and RAMEB/HPBCD were lyophilized at -50°C in a lyophilizer (Labconco freeze dry systems / Freezone 2.5). The freeze-dried powders of the THC-HS: RAMEB/HPBCD complex were used for NMR and FT-IR studies.

3.3.1 Phase solubility studies

The phase solubility study was performed by employing a previously reported procedure by Higuchi.[186] Briefly, THC-HS:RAMEB/THC-HS:HPBCD complexes in aqueous solutions were prepared by adding an excess amount of THC-HS to RAMEB/HPBCD solutions of different concentrations (10mM-200mM). The suspensions were equilibrated for 24 h at 25°C at 100 rpm under constant shaking. After equilibration, undissolved THC-HS was removed from the suspensions by centrifugation. Intrinsic solubility (S_0) of THC-HS in pure water was determined by following the same protocol, but without addition of CDs. All of the samples were prepared in triplicate. The concentration of THC-HS in the inclusion complexes was determined by HPLC assay. Stability constants for the formation of inclusion complexes between THC-HS and RAMEB were determined from the phase-solubility data using the equation (1):

$$K_s = \text{slope}/S_0\ (1\text{-slope}) \qquad (1)$$

3.3.2. NMR spectroscopy

The ^{1}HNMR spectra were recorded at 25°C on a Bruker 400 MHz spectrometer using a 5 mm NMR probe. Since the aqueous solubility of THC-HS is extremely low, the spectra had to be recorded in deuterated water: methanol (1:1). For proton NMR, 128 scans were recorded with the following parameters: 32k data points, pulse width of 4.0μs and relaxation delay of 1s. Digital zero filling to 64K and a 0.5Hz exponential were applied before Fourier Transformation. ^{1}HNMR chemical shifts variations (Δδ) were calculated according to the equation (2)[187,188]:

$$\Delta\delta = \delta \text{ (complex)} - \delta \text{ (free)} \qquad (2)$$

3.3.3. Job's plot

The job's plot of THC-HS was determined from ^{1}H NMR and UV data, according to the continuous variation method.[189] The NMR experiment was carried out with solutions of THC-HS and RAMEB/HPBCD in deuterated water: methanol (1:1). The total molar concentrations of THC-HS and RAMEB/HPBCD were kept constant at 20mM, but their mole fractions were varied. The chemical shift in the proton signals due to the complex formation was plotted against the mole fraction of the two components. Solutions of the same composition, but in unbuffered water only, were used for UV-spectrophotometric determination of the stoichiometry. In this case, the shift of λ_{max} of the UV spectrum of THC-HS was plotted against the mole fraction of the two components. Spectra were obtained with a Shimadzu UV-Vis spectrophotometer.

3.3.4. Fourier Transform Infrared Spectroscopy

The FT-IR spectra were acquired for the lyophilized powders of THC-HS:RAMEB/HPBCD complexes (1:1). FT-IR spectra were recorded using a universal attenuated total reflection sampling accessory with a Zinc Selenide crystal on a Perkin-Elmer Spectrum 100 FT-IR spectrometer. The results were the means of 3 determinations. The physical mixtures of drug alone and RAMEB /HPBCD were used as blank.

3.3.5. Molecular Modeling

Molecular modeling studies were carried out using Maestro molecular modeling suite (Schrödinger Inc.). The three-dimensional structure of β-CD was obtained from the protein complex of alpha-amylase (pdb id:1jl8.pdb) available from the protein data bank. The CD was then extracted and dimethylated using Maestro molecular modeling suite (Schrödinger, LLC, New York, NY, 2005). Modified CD *aka* dimethyl-β-CD (DIMEB) was then energy minimized to remove the steric hindrance and clashes that can appear in the building process due to addition of two methyl groups. DIMEB was adopted as a substitution for RAMEB to facilitate modeling studies.[190] A similar protocol was used to generate the three-dimensional structure of HPBCD. The structure of THC and THC-HS were sketched and energy minimized using Maestro. Molecular docking experiments were carried out using Glide (Glide, version 4.0, Schrödinger, LLC, New York, NY, 2005) module of Schrödinger molecular modeling suite. Glide searches for favorable interactions between small molecules (ligand, THC-HS in this case) and a receptor molecule (DIMEB/HPBCD in this case). The flexible docking procedure using Glide automatically generates conformations for each input ligand. Each conformer generated is then positioned into the cavity of DIMEB/HPBCD to find the preferred binding geometries. The most

likely conformation of the complex was the one with the lowest energy. The parent THC structure was also used in docking studies for comparison with THC-HS. The docked conformation of the THC and THC-HS were merged into the DIMEB and/or HPBCD. The resultant complexes were then minimized using OPLS2002 force field using Macromodel module of Maestro molecular modeling suite. The minimized energy of the complex represents $E_{(complex)}$. The binding energy (ΔE) upon complexation between THC-HS and DIMEB/HPBCD calculated for the minimum energy structure is defined in Eq. (3)[191]:

$$\Delta E = E_{(complex\ )} - (E_{THC-HS} + E_{CD}) \qquad (3)$$

where, $E_{(complex)}$ represents minimized energy of the complex. $E_{THC\text{-}HS}$ and E_{CD} represent the energies of the guest (THC-HS) and host (CD) for the configuration extracted from the optimized complex geometry. The magnitude of the energy change would be a sign of the driving force towards complexation. The more negative the binding energy is the more thermodynamically favorable is the inclusion complex.

3.4. Results and Discussion

3.4.1. Phase Solubility studies

The apparent solubility of THC-HS increases linearly with increasing concentrations of RAMEB as well as HPBCD. The aqueous solubility of THC-HS was increased one hundred-fold from an intrinsic aqueous solubility of 0.092mM to 9mM, in the presence of 0.18 mM RAMEB.

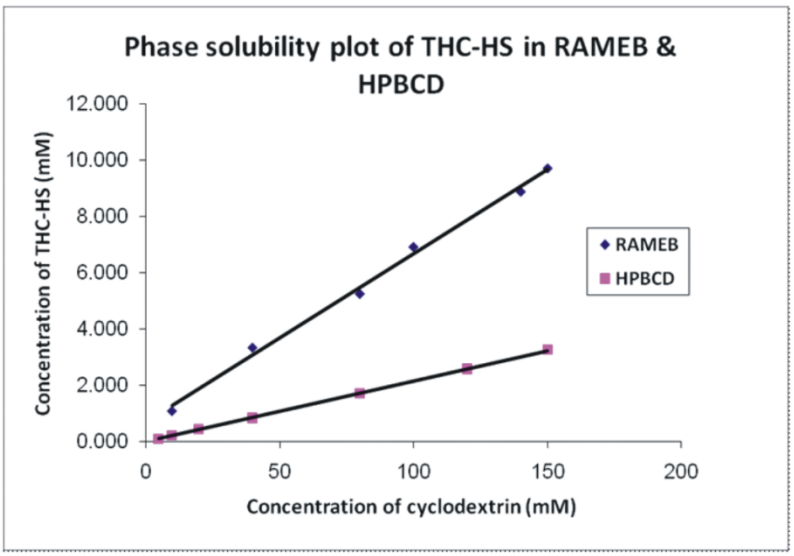

Figure 3-3: Phase solubility plot of THC-HS (RAMEB and HPBCD)

The phase solubility plot of THC-HS with RAMEB and HPBCD is shown in Fig. 3-3.The slope of the phase solubility diagram is smaller than unity, over the entire concentration range studied, indicating an A_L type diagram as seen in Fig. 3 with the formation of a complex with 1:1 stoichiometry.

Table 3-1: Solubility and association constants of THC-HS and THC (mM) in RAMEB and HPBCD

Compound	**Solubility (mM)**		**K (1:1) M^{-1}**	
	RAMEB (150mM)	**HPBCD (150mM)**	**RAMEB**	**HPBCD**
THC-HS	8.088 ± 0.65	3.24 ± 0.89	562.48 ± 5.2	238.83 ± 4.3
THC	5.63 ± 0.9	4.49 ± 1.2	21137.44 ± 15.6	15589.88 ± 23.0

The calculated association constant ($K_{1:1}$) from Table I for the THC-HS–RAMEB complex is 562.48 M^{-1} indicating that THC-HS:RAMEB complexes (1:1 molar ratio) are moderately stable. Actually, lower values of $K_{1:1}$ indicate a weak interaction between drug and CD, while higher values mean an incomplete drug release from the inclusion complex. The association constant with HPBCD (238.83M^{-1}) is lower than that obtained with RAMEB. The higher solubilization in the case of RAMEB is attributed to the presence of hydrophobic methyl groups which extend the hydrophobic cavity of the CD molecule. The values of the association constants of THC-HS indicate a moderately strong complex with RAMEB and a weak complex with HPBCD. The association constant for the parent THC molecule with RAMEB and HPBCD were determined to be 21,137.44 M^{-1} and 15,589.88 M^{-1} respectively. These values were in agreement with those reported by Manilla *et al.*[192] The higher association constants of CDs with THC are indicative of a tighter binding with the CDs and thus lower free drug concentration.

3.4.2. NMR spectroscopy

NMR studies with RAMEB are not without difficulty because any collection of modified-beta-CD molecules is a highly complex mixture of different isomeric forms of variously substituted beta-CD derivatives. Hence only several of the NMR-signals for THC-HS could be unambiguously identified as shown in Fig.3-4.

The signals from RAMEB were inconclusive and could therefore not be interpreted with confidence. Therefore, the prediction of the orientation of THC-HS in the complex with RAMEB/HPBCD is a fair approximation using an array of different techniques to make definite conclusions. In ^{1}H -NMR, significant changes in chemical shifts of protons of the free acid group of the hemisuccinate side chain of THC-HS as well as the protons of the phytyl side chain and

the aromatic ring of THC-HS were observed. Especially the protons of the side chain were found to shift to the maximum extent followed by the carboxylic acid proton indicating that the hydrogen bonding and van der waals interactions are involved in the inclusion complex formation.

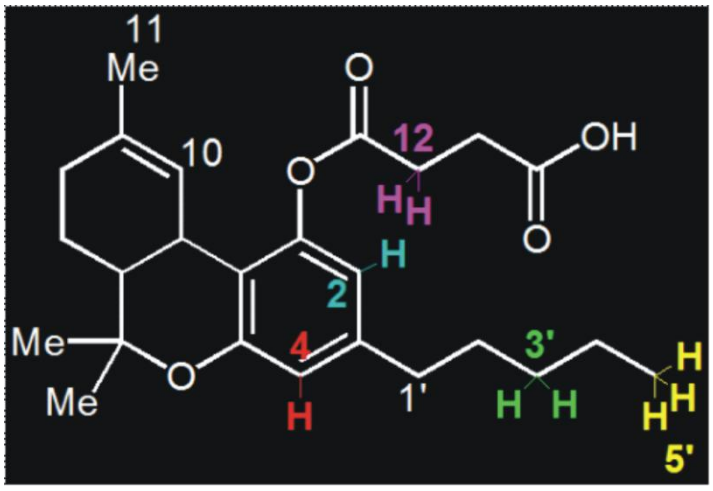

Figure 3-4: Structure ***of*** THC-HS (Protons showing significant changes in chemical shifts shown in different colors)

It was predicted that the molecule could be entering the CD cavity with its aliphatic side chain inserted into the cavity followed by the aromatic ring. The largest chemical shift in signals of H2, H4 H3', H5′ and H12 for THC-HS were observed while that of H7, H8 and H11 remained unaffected (Fig. 3-4). These data indicate an inclusion of ring A and the alkyl side chain of THC-HS into the RAMEB cavity. This may be explained in that the alkyl side chain completely enters the CD cavity and protrudes from the opposite end, exposing H5′ to the solvent. Protons in the ring C were mostly unaffected indicating that the C ring might not be included in the hydrophobic cavity of RAMEB. This is also in good agreement with the observations of Hazekamp et al[188] who reported non inclusion of ring C of THC in RAMEB. Aromatic protons at H2 and H4 undergo an expected upfield shift upon inclusion in the complex. The hemisuccinate

H12 proton exhibited a downfield shift possibly due to its interaction with the solvent. The relatively small Δδ values observed for all signals indicate a relatively weak association.

3.4.3. Job's Plot

Several techniques such as DSC, IR and UV-Vis spectroscopy can establish whether guest molecules form an inclusion complex with CDs, however they cannot provide information about the structural configuration of the complex.[193,194] In contrast, NMR is a technique that provides the most useful evidence for the inclusion of a guest into the hydrophobic CD cavity in solution. Two different techniques were used for preparation of a Job's plot in order to determine the stoichiometry of the inclusion complex. Thus, the ratio of RAMEB/HPBCD and THC-HS was varied, while the sum of their concentrations was kept constant, and a continuous variation plot was prepared. The chemical shift in the proton signals in NMR / shift of λmax due to the complex formation in UV spectroscopy were plotted against the mole fraction of the two components (Fig. 3-5 & Fig. 3-6).

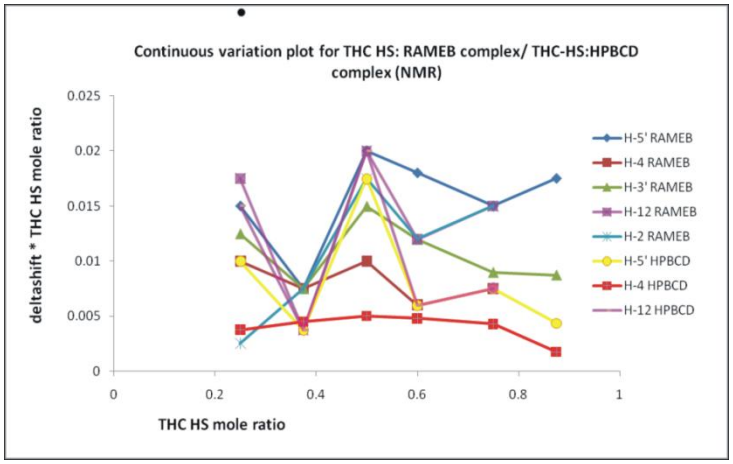

Figure 3-5: Job's plot (NMR spectroscopy)

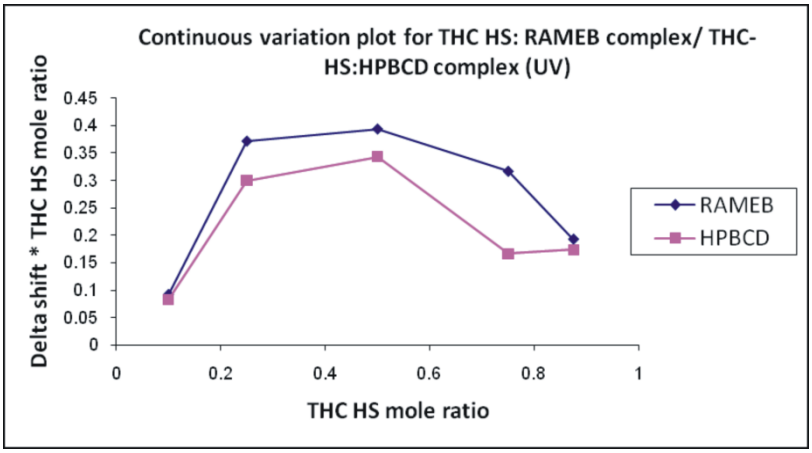

Figure 3-6: Job's plot (UV spectroscopy)

Using this method the value for Δδ reaches a maximum at the stoichiometric point. The results from UV as well as NMR spectroscopy yielded a 1:1 stoichiometry of THC-HS to RAMEB/HPBCD. In a linear 1:1 stable complex, the plot usually has a triangular form with a maximum, while the formation of weak complexes results in curved plots indicating that the complex formed between THC-HS and RAMEB/HPBCD is not a strong one. The values obtained of association constants also suggest the same.

3.4.4. Infrared Spectroscopy

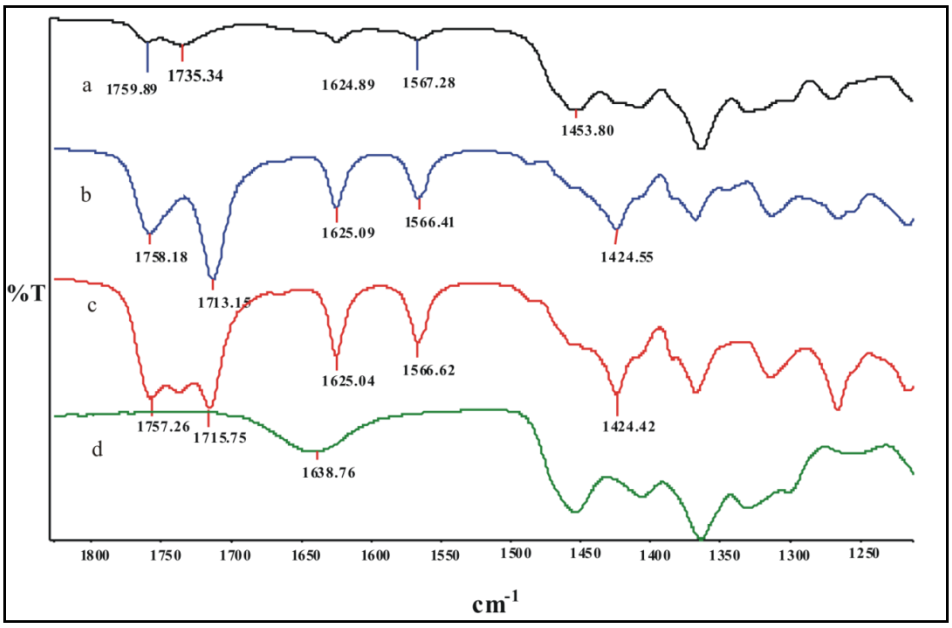

Figure 3-7: FT-IR spectra of Lyophilized complex of THC-HS &RAMEB (a), THC-HS (b), physical mixture of THC-HS &RAMEB (c) and RAMEB (d)

THC-HS was characterized by bands around 1700-1500cm^{-1} and 1300-1100cm^{-1} (Fig. 3-7). The FTIR spectra of CDs showed intense bands at 3600-3200 cm^{-1}, corresponding to absorption by hydroxy groups. The bands that appeared at 3000-2800 cm^{-1} were assigned to stretching vibrations of the bonds in –CH and –CH_2 groups. In THC-HS spectra, C=O stretching vibrations, (1758cm^{-1}, 1713cm^{-1}), -CH_2 bending vibration (1424cm^{-1}) were used to assess the interaction between RAMEB and the guest molecule (THC-HS) in the solid state. The spectra of the physical mixture as well as the lyophilized mixture did not show any new peaks indicating that no new chemical bonds were created in the formed complex. In the physical mixtures, the spectra were the exact superposition of those of the pure compounds, except that the intensities of the peaks had reduced to some extent (Fig. 3-7). However, in the spectra of inclusion complexes, especially with RAMEB (Fig. 3-7), the bands arising from all of the major

interactions like C=O stretching vibrations, (1758cm^{-1}, 1713cm^{-1}) and -CH_2 bending vibration (1424cm^{-1}) have decreased in intensity and moved toward the left. This is likely due to the restrictions in the vibrations from hydrogen bonding during CD complexation. The bands located at 1758cm^{-1} and 1713 cm^{-1} are correlated to the stretching vibrations of the carbonyl group of the ester and the free carboxylic acid, respectively. In the lyophilized complex of THC-HS and RAMEB, the band from the free carboxylic acid at 1713 cm^{-1} (shifted to 1735 cm^{-1} in lyophilized complex) has broadened and decreased in intensity due to the potential hydrogen bonding. The band from the ester carbonyl group did not show any shift in the wavelength upon complexation but was visible at a very low intensity indicating that the hydrogen bonding interactions with RAMEB are primarily occurring at the free carboxylic acid end of the guest molecule (THC-HS). Another important interaction observed at 1424cm^{-1} is –CH_2 bending vibrations. This band is also minimized in intensity as it relates to the hydrocarbon chain of THC-HS. As compared to RAMEB, the HPBCD inclusion complex of THC-HS did not show any significant interaction and the differences in the IR spectrum of the physical mixture and lyophilized complexes were too subtle to be identified. It is likely that the molecule enters the CD molecule with its hydrocarbon tail inserted into its hydrophobic cavity first with the hemisuccinate ester side chain in close proximity to the rim hydroxyl groups of RAMEB. These results support the assumption that the inclusion complex was formed when the lyophilization was used in contrast to the physical mixture of THC-HS and RAMEB/HPBCD (Fig 3-7). FT-IR spectrum for THC-HS HPBCD complex could not be clearly deciphered due to the low binding affinity of THC-HS for HPBCD (Data not shown).

3.4.5. Molecular Modeling

The docking studies indicated that the aromatic ring and the appended hydrophobic phytyl chain of the THS-HS molecule are oriented inside the cavity of DIMEB (Fig. 3-8).

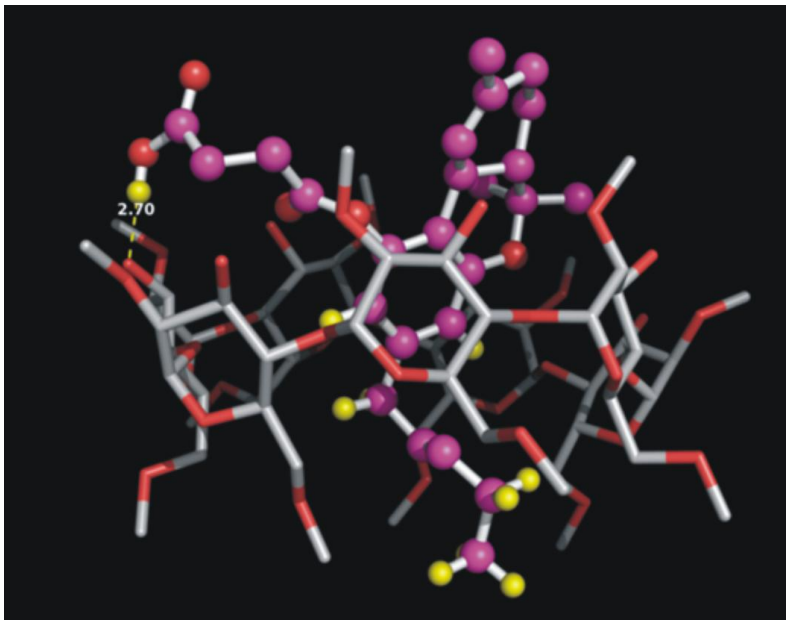

Figure 3-8. Binding pose of THC-HS within DIMEB cavity during formation of THC-HS:RAMEB complex, as predicted by Glide docking program.

From this figure, the tricyclic ring of THC-HS shows van der waals interactions with the hydrophobic cavity of DIMEB. It is quite significant to note that no fixed constraints were imposed during the docking calculations and the docking results corroborated with the data obtained from NMR and FT-IR spectroscopy. The terminal carboxylic acid functional group of the hemisuccinate ester side chain is involved in hydrogen bonding interactions with one of the hydroxy groups on the surface of DIMEB. The NMR results demonstrated changes in the chemical shift values of a few protons, including the aromatic and the phytyl chain protons. As can be further seen from Fig. 3-8, the protons shown in yellow are submerged inside the DIMEB cavity. Similarly, IR studies showed shifting of a carboxylic acid stretch due to hydrogen

bonding interactions with one of the hydroxy groups of CD. The docking studies revealed that the carboxyl group of the hemisuccinate side chain lies within hydrogen bonding distance from the hydroxy of DIMEB (2.7Å).

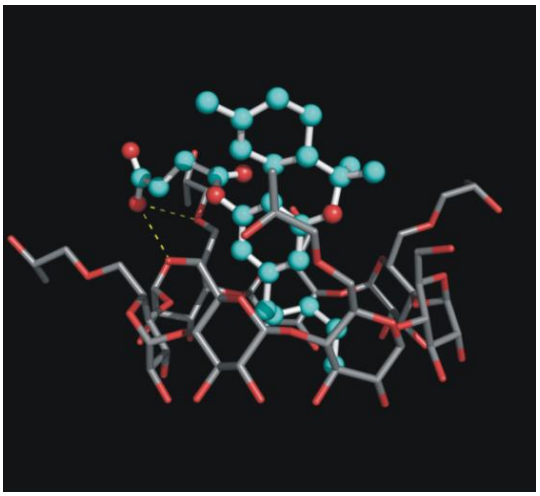

Figure 3-9: Binding pose of THC-HS within HPBCD cavity during formation of THC-HS:HPBCD complex, as predicted by Glide docking program

The docking studies of THC-HS with HPBCD demonstrated a lower binding affinity of the ligand for HPBCD (Fig. 3-9).

Table 3-2: Binding energies and docking scores of the inclusion complexes of THC-HS and THC

Complex ID	Binding Energy	Van der Waal Energy	Docking score
THC-HS - DIMEB	-182.9	-66.69	-5.42
THC-HS - HPBCD	-125.23	40.43	-2.96
THC-DIMEB	-137.74	-42.47	-5.65
THC- HPBCD	-125.07	40.43	-3.33

In Table 3-2, the binding energies, along with the van der Waals energies and the docking scores of the various inclusion complexes of THC-HS and THC with DIMEB and HPBCD are provided. THC and THC-HS showed similar docking scores (-5.42 and -5.67). Hence, the binding energy calculations were performed to understand the stability of the two complexes. It is evident that the THC-HS showed better binding and thus complexation (ΔE = -182.9 kJ/mol) with DIMEB compared to the parent molecule THC (ΔE = -137.73 kJ/mol). THC-HS: DIMEB complex shows the lower binding energy and docking score compared to the THC-HS: HPBCD complex, suggesting that the later is energetically less favored. This data is also supported by strong van der Waals interactions reflecting the snug fit of THC-HS obtained while complexation with DIMEB (-66.69 kJ/mol) as compared to HPBCD (-40.43 kJ/mol) When compared with THC, the binding energy of the THC: DIMEB complex (BE = -137.74 kJ/mol) is significantly higher than that of the THC-HS: DIMEB complex (BE = -182.9 kJ/mol).[195] Higher the binding energy, lower the binding affinity and hence the lower solubility too. THC-HS thus showed higher solubility than THC in DIMEB. The molecular modeling results correlated very well with the phase solubility and NMR spectroscopic data in that RAMEB was able to better solubilize THC-HS as compared to HPBCD. The best possible mode of binding of THC-HS with DIMEB is shown in Fig. 3-10.

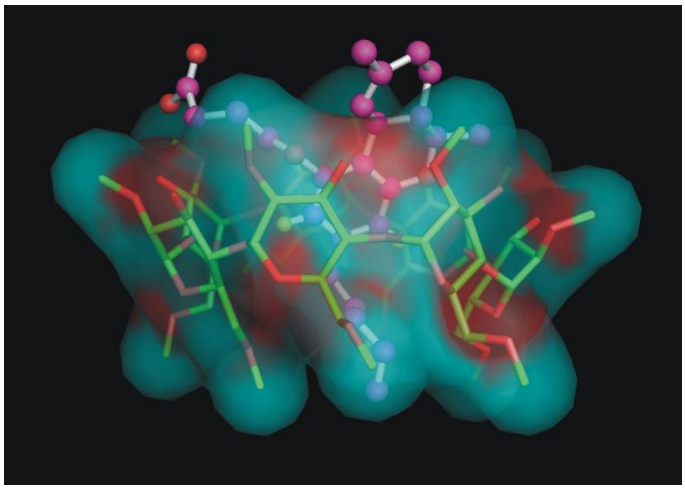

Figure 3-10: Orientation of THC-HS molecule in DIMEB cavity, the polar portion of THC-HS oriented outside the cavity, as predicted by Glide docking program

From this figure, it is very clear that the alkyl chain is embedded in the hydrophobic cavity of DIMEB whereas the hemisuccinate ester side chain forms hydrogen bonding interactions with one of the surface hydroxyl groups of the DIMEB molecule.

3.5. Conclusion

Inclusion complexes of THC-HS with various modified CDs were prepared by lyophilization and characterized using several techniques such as phase solubility, NMR, FT-IR spectroscopy and molecular modeling. The aqueous solubility of THC-HS was increased by almost 100-fold due to the inclusion complex formation with RAMEB. HPBCD was unable to form a stable complex with THC-HS and subsequently enhance its solubility in aqueous medium. The results obtained from phase solubility and Job's plot suggests the formation of a 1:1 inclusion complex of THC-HS and RAMEB. NMR and FT-IR spectroscopy were useful in characterization of the structure of the inclusion complex formed. Higher association constants were obtained and in turn, better solubilization of THC-HS was achieved by RAMEB as

compared to HPBCD. Our experimental data was well-supported by molecular modeling studies suggesting energetically favorable inclusion complex formation of the THC-HS: RAMEB complex as compared to the THC-HS: HPBCD complex. The complex formation of THC-HS with RAMEB and HPBCD predicted by molecular docking studies corroborated well with the results of NMR and FT-IR spectroscopy. CDs have aided in solubilization of THC-HS in vitro and the solid dispersions of THC-HS and RAMEB will be further incorporated into solid dosage forms for development of stable oral controlled release THC formulations.

Acknowledgements

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CHAPTER 4

Chemical stability and Enzymatic hydrolysis of complexes of a hemisuccinate ester of Δ^9-THC in presence of modified cyclodextrins

4.1. Introduction

Cyclodextrins (CDs) are water-soluble naturally occurring cyclic glucose oligomers which have the ability to include selectively a range of guest species in their hydrophobic cavity. The formation of an inclusion complex potentially alters the physical and chemical properties of the guest molecule. The binding forces associated with the CD-guest inclusion formation are attributed to a number of factors such as hydrophobic forces, hydrogen bonding, size of the cavity, shape of the guest molecule and electrostatic interactions.[196,197]Several previously published reports have documented the use of cyclodextrins to improve solubility, stability and bioavailability of drugs. Recently, we published solubilization studies of a hemisuccinate ester of Δ^9-Tetrahydrocannabinol (THC-HS) with modified cyclodextrins.[197] (Δ^9-Tetrahydrocannabinol (THC) is the primary active ingredient of the plant *Cannabis sativa* (marijuana) and is responsible for the majority of the pharmacological effects. While THC in marijuana is mainly known for its abuse potential, it also exhibits therapeutic effects in the treatment of nausea and vomiting during cancer chemotherapy, in appetite stimulation, cachexia associated with AIDS, glaucoma, analgesia, and other indications.[163] To date, the most promising clinical applications approved by the Food and Drug Administration (FDA) are for the control of nausea and vomiting associated with chemotherapy and for appetite stimulation of AIDS patients suffering from anorexia and wasting syndrome. Orally administered THC from Marinol® soft gel capsules has shown slow and variable absorption due to low oral solubility/permeability and first pass metabolism.

The approach of synthesizing more hydrophilic prodrugs of the molecule has been successfully used in modifying the physico-chemical properties of the parent drug without changing the core structure of the compound. Such reversible derivatization of the parent

molecule helps in improving its pharmacokinetic properties and drug delivery characteristics. Hence we have designed hydrophilic prodrugs of THC such as THC-HS to overcome the pharmacokinetic limitations of the parent molecules of the parent molecule, THC.

The purpose of this study was to investigate the effect of cyclodextrins *viz*.; RAMEB and HPBCD on chemical and enzymatic stability and *in vitro* buccal permeation of THC-HS. This strategy would allow an increase in aqueous solubility without a change in the molecular structure and the intrinsic ability of the lipophilic esters to partition into biological membranes. Cyclodextrins are cyclic oligosaccharides with a hydrophilic outer surface and a hydrophobic central cavity. The hydrophilic exterior renders the cyclodextrin water-soluble and the hydrophobic interior provides a microenvironment for relatively non polar drugs. In aqueous solutions, cyclodextrins can form inclusion complexes with lipophilic drugs by entrapping either the entire drug molecule or a non polar part of it inside the hydrophobic cavity. Such encapsulation may protect the prodrug against potential degradation by buccal membrane–bound and salivary esterases.[38]

4.2 Materials

Monobasic potassium phosphate, monobasic sodium phosphate, dibasic sodium phosphate and dibasic potassium phosphate were purchased from Spectrum Chemical, Inc., (Gardena, CA). Lithium chloride, hydrochloric acid (HCl), sodium hydroxide (NaOH), potassium hydroxide, sodium chloride, potassium sulfate, methanol and acetonitrile (both HPLC grade) were obtained from Fischer Chemicals (Fair Lawn, NJ). HPLC grade water was freshly prepared in the laboratory (by Nanopure system, Barnstead, Dubuque, IA)

4.3. Methods

4.3.1. Chemical Stability Studies

Stability of THC-HS at 25°C in three different pHs (3, 5 and 7.4-phosphate buffer), in the absence and presence of RAMEB/HPBCD (1% w/v) was determined. A prodrug stock solution (100 μL) in ethanol was subsequently added to the 1 ml of buffer solutions to yield a concentration of 10 μg/ml. The vials were placed in a constant shaker bath set at 25°C and 60 rpm. Samples (100 μL) were collected at appropriate time intervals for up to 48 hours and stored at –80°C until further analysis. Linear regression of the log concentration versus time profiles yielded the pseudo first order rate constants of degradation. Degradation studies were also carried out using 1% RAMEB in pH 7.4 phosphate buffer saline, to study the effect of cyclodextrin on chemical stability of the prodrugs.

4.3.2. Enzymatic Hydrolysis Studies

Initially, protein estimation of buccal tissue homogenates was performed using Bradford reagent. Hydrolysis studies were carried out in the presence and absence of RAMEB/HPBCD (1% w/v) to determine if cyclodextrin complexation can increase the stability of THC-HS against enzymatic degradation by membrane-bound enzymes of the buccal mucosa. Ethanolic stock solutions of THC-HS:RAMEB/HPBCD were added to porcine buccal tissue homogenates in pH 7.4 buffer at 25°C. Samples were withdrawn every 15 minutes for the first hour and every 30 minutes for the next 2 hours, and the samples were subsequently stored at –80°C until further analysis was performed. Studies were performed in triplicate. The solutions were analyzed for THC-HS content.

4.3.3. *In Vitro* Permeability Studies

Buccal membrane permeation studies were carried out using stripped porcine buccal mucosa. The porcine buccal mucosa was prepared by heat-stripping a piece of porcine jaw in water at 60°C according to the standard procedure. Immediately following excision, the mucosa was washed with ice-cold pH 7.4 buffer and mounted on side-by-side diffusion chambers with the epithelial side facing the donor compartment. The integrity of buccal mucosa was determined by electrical resistance apparatus. The temperature was maintained at 32°C. The donor compartment consisted of (a) aqueous solutions of THC-HS in presence of 0.1% SLS and 0.5% Cremophor RH 40 and (b) aqueous solutions of THC-HS in presence of 1%w/v RAMEB. The receiver compartment consisted same solutions as that of the donor compartment except THC-HS. The donor and receiver compartments were stirred continuously with magnetic stir bars. The total duration of the transport was 8 hours and samples (600µl) were withdrawn from the receiver compartments for 30 minutes in the first hour and every hour in the next 7 hours. The samples were immediately replaced with an equal volume of receiver solution to ensure sink conditions. The samples were analyzed for THC-HS as well as regenerated THC by HPLC. All the experiments were conducted in triplicates.

4.4. Results and Discussion

4.4.1. Chemical stability studies

One of the most common pharmaceutical applications of cyclodextrins is to enhance drug stability in aqueous solutions. Inclusion complex formation may be regarded as an encapsulation of the prodrug molecule or at least a labile ester segment of the molecule. Entrapment, partial or total can protect the prodrug against attack by various reactive species. This ability to form

inclusion complexes has been exploited to alter the chemical and physical properties of guest (drug) molecules, to improve water solubility, prolong in vivo stability, reduce toxicity and irritancy, and improve bioavailability.[38]

Release of the guest molecule is governed by dissociation, and therefore selective chemical modifications can be employed to control equilibrium thermodynamics, and thus release rate.

Table.4-1. lists the degradation rate constants of the complexed as well as free prodrug at 37ºC in pH 3, 5, 7.4 phosphate buffer. There was a significant reduction in chemical hydrolysis of complexed prodrug as compared to free prodrug. RAMEB afforded better stability profile and lower degradation rate constants (fig.4-1 & Table 4-1) as compared to HPBCD at all the pHs tested. The effect was more pronounced at pH 5 where the prodrug has better stability profile due to its acidic nature. Complexation with RAMEB and HPBCD increased the half-life of THC-HS by approximately 12 fold and 8 fold respectively. The higher stability is achieved with THC-HS having higher complexation constant with RAMEB as compared to HPBCD.

THC-HS exhibits maximum stability between pH 3.0 and 5.0 and lower stability at pH 7.4. The half life of THC-HS in RAMEB solutions increased to 55 hours and 17.5 hours at pH 3 and 7.4 respectively as compared to 9 hours and 1.5 hours at same pHs for the uncomplexed prodrug. It was shown earlier that THC-HS exhibited maximal stability at pH 3-4.5. However,, decreasing the pH of the dosage form to drastic levels like pH 3 may lead to enhancement in buccal irritation. Hence moderately enhanced stability of the complexed prodrug between pHs 5 and 7.4 will definitely prove beneficial in formulation of transmucosal films with sufficient patient compliance.

Increased stability of the prodrugs in aqueous solutions of RAMEB/HPBCD solutions indicated that the ester moiety is at least partially enclosed in the cyclodextrin cavity. It can be estimated that the ester linkage might be protected by either insertion in to the hydrophobic cavity of RAMEB/HPBCD or by forming hydrogen bonds with its rim hydroxyl groups. [59]

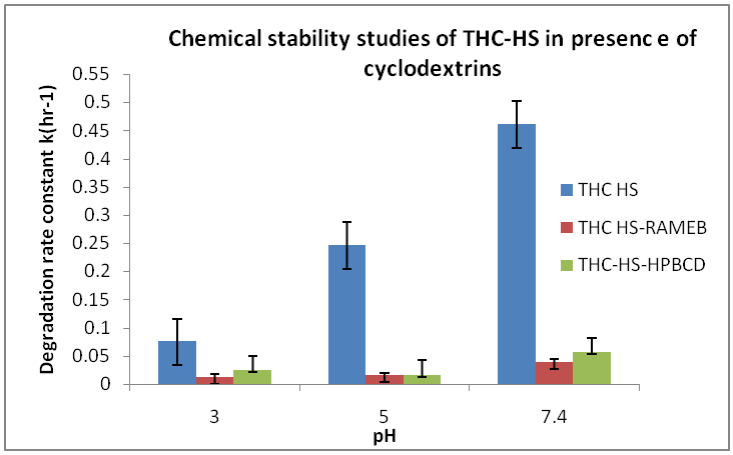

Figure 4-1: Chemical stability of THC-HS in presence of RAMEB & HPBCD

Table 4-1: Chemical stability of THC-HS in presence of Cyclodextrins

Compound	pH		
	3.0	**5.0**	**7.4**
THC-HS	0.076±0.013	0.246±0.04	0.462±0.043
THC-HS:RAMEB	0.0124±0.0489	0.0158±0.0125	0.0395±0.0047
THC-HS:HPBCD	0.0242±0.0125	0.0165±0.0035	0.0564±0.026
	$T_{1/2}$ (hrs)		
THC-HS	9.118±0.013	2.805±0.04	1.5±0.043
THC-HS:RAMEB	55.887±0.0489	43.860±0.0125	17.5443±0.0047
THC-HS:HPBCD	28.636±0.0489	43.3125±0.0035	12.2873±0.026

4.4.2. Enzymatic hydrolysis

In this study, in order to improve the stability and bioavailability of prodrug THC-HS, we investigated the effect of RAMEB and HPBCD on the enzymatic hydrolysis of THC-HS. In buccal homogenate studies, at a protein concentration of 0.5 mg/ml, enzyme mediated hydrolysis of THC-HS was decreased 3-folds in the presence of RAMEB in pH 7.4 phosphate buffer. The degradation rate constant was 0.0079 min^{-1} for THC-HS:RAMEB complex compared to a degradation rate constant of 0.023min^{-1} for THC-HS alone (fig.4-2 & Table 4-2). THC-HS:HPBCD complex demonstrated a lower stability towards enzymatic hydrolysis with a degradation rate constant of 0.0114 min^{-1}. It is apparent from the complex $t_{1/2}$ values that RAMEB produced the least labile complex. The alkaline hydrolysis of THC-HS was considerably slower in presence of RAMEB and HPBCD. The degree of stabilization by CD complexation depends on both the hydrolysis rate of free THC-HS within the solution and within the complex. THC-HS had a much higher complexation constant with RAMEB than HPBCD which increased the fraction of complexed prodrug in the solution, thereby resulting in greater stabilization with RAMEB than HPBCD. A stronger association was observed for RAMEB than for HPBCD. RAMEB which has secondary hydroxyls of each glucopyranose unit, and primary hydroxyls, methylated, was more efficient in the improvement of stability of nonpolar part of THC-HS. The methyl groups confer hydrophobicity close to the extremity of the hydrophobic cavity of the cyclodextrin and greater flexibility to its structure. This yields a higher association constant. In case of HPBCD, the bulky hydroxypropyl groups on primary hydroxyls cause a steric hindrance and, consequently, a lesser association constant and a lesser stabilization effect on THC-HS. The buccal mucosa has been known to contain a high activity of various esterases and peptidases which may limit the absorption of enzymatic labile drugs such as prodrugs

following buccal or sublingual administration.[198] Another absorption-limiting factor for such compounds may be degradation by enzymes in the saliva. Human saliva has been shown to contain a variety of esterases, mainly carboxylesterases and therefore, saliva-catalyzed degradation of ester prodrugs or drugs containing susceptible ester prodrugs may preclude an efficient buccal bioavailability.[131] The catalytic effect of buccal as well as salivary esterases on the hydrolysis of THC-HS is evident by comparing the half-life of complexed prodrug in buccal homogenates with that in a pH 7.4 phosphate buffer at 37ºC.

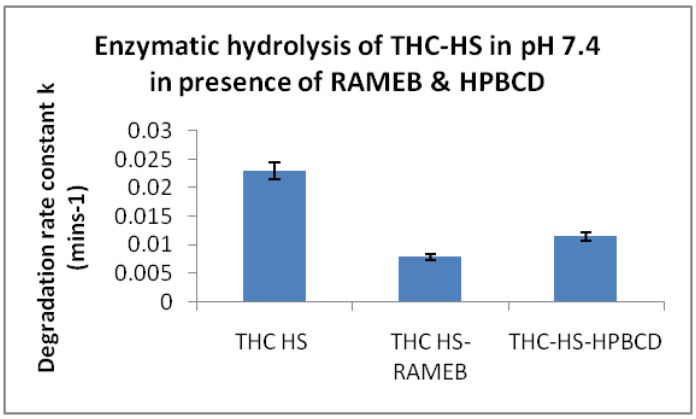

Figure 4-2: Enzymatic hydrolysis of THC-HS in presence of RAMEB & HPBCD

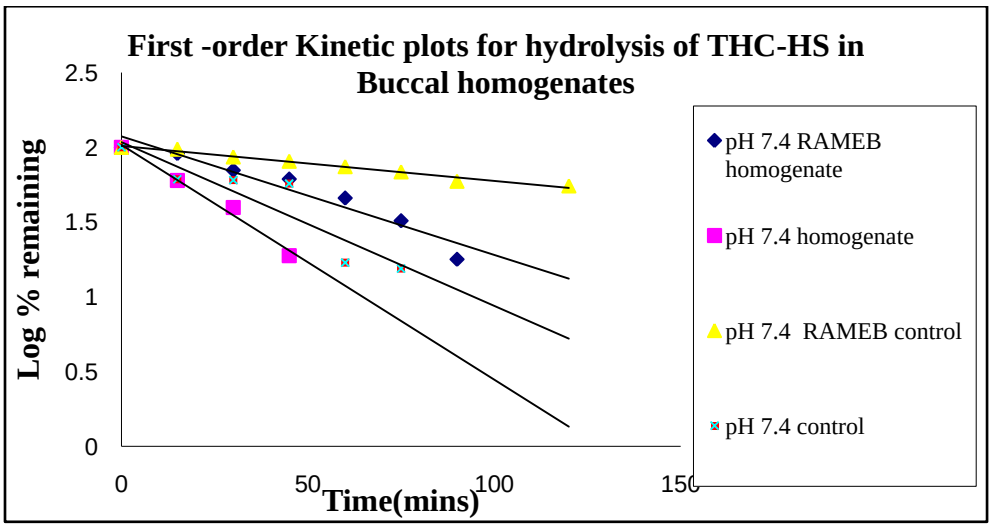

Figure 4-3: First-order kinetic plots for the hydrolysis of THC-HS in buccal homogenates

Table 4-2: Enzymatic hydrolysis of THC-HS in presence of cyclodextrins

Compound	**Degradation rate constant k (min^{-1})**
THC-HS	0.023±0.013
THC-HS:RAMEB	0.0079±0.0489
THC-HS:HPBCD	0.0114±0.0125
	$t^{1/2}$ (min^{-1})
THC-HS	30.13 ±0.013
THC-HS:RAMEB	87.72 ±0.0489
THC-HS:HPBCD	60.78±0.0125

The half–life of complexed prodrug THC-HS with RAMEB has been reduced to approximately 88 minutes as compared to 17.5 hours in pH 7.4 phosphate buffer solution (Tale.4-2). However the complexed prodrug still exhibits a much better enzymatic stability as compared to free prodrug which is evident from its half-lives given in Table. 4-2. The results of the chemical and enzymatic degradation profile indicated that the complexed prodrug hydrolyzed at a much slower rate compared to the uncomplexed or free prodrug.

4.4.3. *In vitro* permeability studies

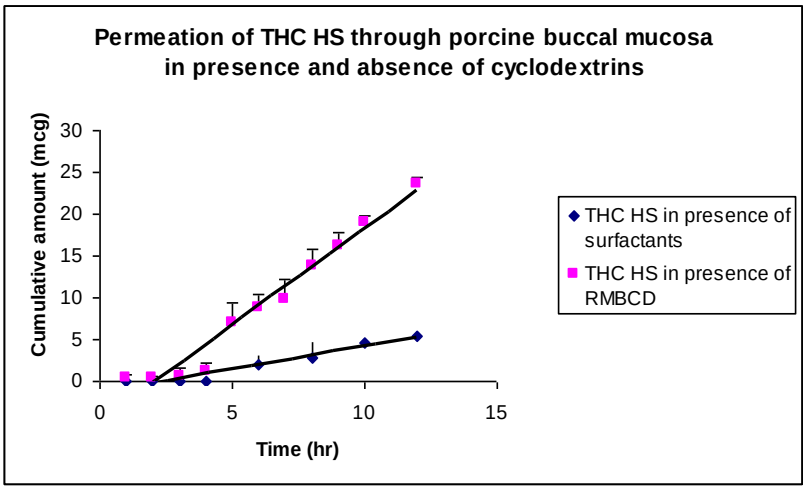

Figure 4-4: *In vitro* permeability studies of THC-HS in presence of cyclodextrins

Buccal mucosa is a potential site for drug absorption in alternative to oral drug delivery. Active molecules administered through the buccal mucosa pass directly into the systemic circulation, thereby minimizing the first hepatic pass and adverse gastro-intestinal effect. However, lower permeability of the buccal mucosa to large molecules can be problematic in order to achieve therapeutic levels of such molecules. Buccal permeation can be increased by using various penetration enhancers.[199] Recently, cyclodextrins have been classified as a new class of penetration enhancers. It is generally recognized that cyclodextrins act as true carriers by keeping the hydrophobic drug molecules in solution and deliver them to the surface of the biological membrane, where they partition into the membrane.[200] Cyclodextrins can enhance drug permeation by increasing drug availability and stability at the surface of the biological barriers. However, derivative cyclodextrins, especially methylated cyclodextrins, act as absorption enhancers by different pathways. These hydrophobic cyclodextrins act as absorption enhancers, probably, by transiently changing membrane permeability, overcoming the aqueous diffusion barrier and opening tight junctions.

Table 4-3: Comparative profiling of permeability parameters Indicated values are means (±SD, n = 3–4)

Systems	P_{app} (cm/s)	Ratio[a]	Flux (µg/cm^2.h)
THC-HS	2.00E-08	1	0.07
THC-HS_Surfactants (0.1%SLS + 0.5%Cremophor RH 40)	2.25E-07	11.23	0.815
THC-HS_RAMEB	1.27E-06	63.33	3.639
THC-HS_HPBCD	6.70E-07	33.5	1.930

[a] Enhancement ratio = P_{app} (sample)/P_{app} (control)

In vitro permeation experiments demonstrated almost 63-fold increase in the permeability of THC-HS across excised buccal mucosa, in the presence of RAMEB (1.27 x 10^{-6} cm.sec^{-1} compared to the permeation of THC-HS in the presence of Cremophor RH 40 and sodium lauryl sulphate (2.25x 10^{-7} cm.sec^{-1}). Moreover, permeation lag time of THC-HS decreased to 2 h, from 6-7 h, in the presence of RAMEB (fig.4-4, & Table 4-3.). Integrity of buccal mucosa was maintained throughout the experiment.

Cyclodextrins enhance the permeation of the drug by carrying the drug through the aqueous barrier towards the surface of the membrane, where the drug passes from the complex into the membrane. Addition of RAMEB to the aqueous solution of THC-HS increased the flux by increasing the solubility of THC-HS, thus improving the diffusible form of the drug species at the buccal membrane interface. Though the complex did not penetrate the membrane, the drug in the complex was in rapid dynamic equilibrium with the "free" drug, thus continuously supplying the drug molecules to the membrane surface in a diffusible form. The stability constant value for the THC-HS:RAMEB complex was found to be 562.48 M^{-1}, indicating a moderately labile

association of THC-HS and RAMEB, which was desirable because only the free drug was in the diffusible form. The stability constant of the complex affected the drug diffusivity. The solubility and stability constant of THC-HS with HPBCD was low and its hydrolysis constant high indicating a less stable and soluble complex than that of RAMEB. Hence lower amounts of THC-HS permeated through the buccal mucosa in presence of HPBCD as compared to RAMEB.

4.5. Conclusion

Cyclodextrins were successful in enhancing the stability of THC-HS by virtue of forming of inclusion complex that serves in protection of hydrolysis of ester linkage of THC-HS. Modified cyclodextrins, especially RAMEB were able to increase the flux and decrease the lagtime associated with the permeation of THC-HS through the porcine buccal mucosa.

Acknowledgements

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CHAPTER 5

Thermal stability and release studies of lyophilized solid dispersions of a hemisuccinate ester of THC with modified cyclodextrins

5.1. Introduction

Over the years, Δ^9-Tetrahydrocannabinol (THC) obtained from *Cannabis sativa* has gained significant importance due to its use as an antiemetic in the treatment of nausea and vomiting in cancer chemotherapy. Marinol® soft gel capsule and two of its generic versions are the only USFDA approved formulations available in the market for the treatment of chemotherapy related nausea and vomiting. Since THC from Marinol® capsule is known to have variable absorption due to its high first pass metabolism, there is a need to develop formulations of THC for alternatives routes. The transmucosal films of THC applied to buccal mucosa would enhance the bioavailability of THC by avoidance of first pass metabolism encountered through oral absorption. THC is known to be a very highly lipophilic and unstable molecule therefore hydrophilic prodrug of THC viz, Δ^9-Tetrahydrocannabinol hemisuccinate (THC-HS) was synthesized to improve the physicochemical and pharmacokinetic properties of the parent molecule, THC. It was also thought that preparation of solid dispersions of THC-HS with cyclodextrins would have two-fold benefits in improving solubility as well as stability of the prodrug in cyclodextrin solution. Solid dispersions consist of a carrier in which a compound is dispersed as small particles. When the compound is molecularly dispersed, the term solid solutions or amorphous glass solutions is used.[201,202] Solid dispersions are also used to accelerate dissolution of lipophilic compounds in the aqueous environment of the gastro-intestinal tract and thereby improving their oral bioavailability. [202-204]

Cyclodextrins (CDs) are versatile pharmaceutical excipients which are frequently used to improve the physicochemical and biopharmaceutical properties of drugs (e.g., solubility, stability, and bioavailability.[205,206] CDs have the ability to include selectively a range of guest species in their hydrophobic cavity by virtue of forming inclusion complexes by encapsulating a

part of or whole molecular entity in its hydrophobic cavity. This molecular encapsulation increases the solubility[207] (Manolikar & Sawant, 2003), chemical stability [208]and absorption of the drug. Several previously published reports have documented the use of cyclodextrins to improve solubility, stability and bioavailability of drugs.

Typically, different types of methods have been used for the preparation of inclusion complexes of drug with CDs. Some of these methods are grinding, slurry complexation, solvent evaporation and coprecipitation. All these method require excessive pharmaceutically unacceptable organic solvents and residual solvents need to be removed. Coprecipitation needs excessive CD and high temperature, and this is not suitable to heat unstable drugs like THC-HS. All these methods are difficult for industrial scaling up. Lyophilization, on the other hand is an industrially applicable method especially for heat labile drugs and biopharmaceutical compounds. It produces solid amorphous dispersions with good handling properties. Recently, we published solubilization studies of a hemisuccinate ester of Δ^9-Tetrahydrocannabinol (THC-HS) with modified cyclodextrins. [197]The purpose of this study is to determine the optimum concentration of RAMEB and HPBCD required for dissolution enhancement and establish the most suitable method for preparing the solid dispersions. The aim of this study was to develop a chemically and physically stable dry powder containing THC-HS with improved solubilization. The stability of lyophilized solid dispersions of THC-HS with RAMEB and HPBCD was determined at different stability protocols to assess the effect of RAMEB and HPBCD on the content of THC-HS in lyophilized solid dispersions. *In vitro* permeation of THC-HS from the lyophilized solid dispersions of cyclodextrins across the porcine buccal mucosa was also studied to correlate the enhancement in solubility of THC-HS to its increased flux across the biological membrane.

5.2. Methods

5.2.1. Preparation of inclusion complexes in solid state

Solid dispersions were prepared with different molar ratios (1:1, 1:2, 1:5) of THC-HS and RAMEB /HPBCD using three distinct methods: physical mixture, coevaporation and lyophilization. Physical mixtures were prepared by adsorbing solution of THC-HS in hexane over the bed of RAMEB/HPBCD. The mixture is air-dried and ground to a fine powder in a mortar. Lyophilized mixtures were prepared by addition of known amount of THC-HS and RAMEB/HPBCD to water and freeze-drying the mixture at -50°C in a lyophilizer. Coevaporated samples were prepared by evaporating the aqueous dispersions of THC-HS and RAMEB under vacuum.

5.2.2. Dissolution studies of solid dispersions of THC-HS:cyclodextrin complexes

Dissolution studies of the solid dispersions were performed utilizing a Hanson SR8-Plus dissolution test system USP 31 apparatus 1 basket method. The dissolution medium consisted of 500 ml of 0.5% SLS. The media were previously filtered, degassed and maintained at 37 ±0.5°C. The stirring speed was set at 50 rpm and the temperature was maintained at 37 ±0.5°C. Aliquots from samples containing 10 mg of THC-HS or its equivalent in physical mixture or inclusion complex form, prepared by coevaporation, adsorption and lyophilization were withdrawn each 15 min for a period of 60 min and analyzed by HPLC. Three replicates have been made for each experiment.

5.2.3. Accelerated stability testing of lyophilized THC-HS:RAMEB solid dispersions

The inclusion complexes of THC-HS:RAMEB was prepared by lyophilization of THC-HS and RAMEB (1:2 and 1:10 ratios). Stability of these solid dispersions was assessed at 4°C, 25°C and 40°C in open and closed vials over a period of 1 month.

5.2.4. *In Vitro* Permeability Studies of lyophilized THC-HS:RAMEB solid dispersions

Buccal membrane permeation studies were carried out using stripped porcine buccal mucosa. The porcine buccal mucosa was prepared by heat-stripping a piece of porcine jaw in water at 60°C according to the standard procedure. Immediately following excision, the mucosa was washed with ice-cold pH 7.4 buffer and mounted on side-by-side diffusion chambers with the epithelial side facing the donor compartment. The integrity of buccal mucosa was determined by electrical resistance apparatus. The temperature was maintained at 32°C. The donor compartment consisted of aqueous solutions of THC-HS:RAMEB complex (1:2 & 1:10). The receiver compartment consisted of 7.4 buffer containing RAMEB (1%w/v). The donor and receiver compartments were stirred continuously with magnetic stir bars. The total duration of the transport was 8 hours and samples (600µl) were withdrawn from the receiver compartments for 30 minutes in the first hour and every hour in the next 7 hours. The samples were immediately replaced with an equal volume of receiver solution to ensure sink conditions. The samples were analyzed for intact THC-HS as well as regenerated THC by HPLC. All the experiments were conducted in triplicates.

5.3. Results and Discussion

5.3.1. Dissolution studies of solid dispersions of THC-HS:cyclodextrin complexes

It is interesting to note that the physical mixture of THC-HS with RAMEB and HPBCD does not contribute significantly to an improvement of drug dissolution rate. The possible explanation for this behavior is the non-formation of inclusion complex between the prodrug and the cyclodextrins. The coevaporated samples showed an enhanced dissolution rate which was much better than that obtained by physical mixtures of THC-HS and cyclodextrins. But the coevaporated samples suffered from handling difficulties along with the loss of drug due to the formation of sticky mass instead of free-flowing powders. The release of the THC-HS from lyophilized solid dispersions with RAMEB and HPBCD was enhanced as compared to the physical mixtures as well as co-evaporated samples. At 90 minutes, 90% of THC-HS was released from the 1:1 lyophilized RAMEB complex as against 67% from the adsorbed physical mixtures and 50% from the co-evaporated mixtures (fig. 5-1). At the end of 30 minutes, the percent drug dissolved from the control (THC-HS) was only 14% while percent drug dissolved from 1:5 lyophilized dispersions of THC-HS:RAMEB and THC-HS:HPBCD was 90.3% and 72.0%, respectively. An increase of THC-HS:RAMEB molar ratio from 1:1 to 1:5 increased the dissolution of THC-HS from 66% to 90.3% at the end of 30 minutes (fig. 5-2). At the highest concentration i.e. 1:5 ratio of THC-HS:HPBCD (fig 5-4), at the end of 30 minutes only 72% drug was released as against 90.36 % from RAMEB lyophilized solid dispersions. RAMEB was significantly more effective than HPBCD in improving the dissolution rate of THC-HS from the lyophilized as well as co-evaporated solid dispersions. The physical mixture as well as coevaoprated sample were found to be an inappropriate method of producing solid dispersions of THC-HS:RAMEB/HPBCD as the drug produced sticky, glassy dispersions which were difficult

to handle (fig. 5-3). Incomplete release was found from the physical mixtures of THC-HS:RAMEB and THC-HS:HPBCD even after three hours of dissolution testing.

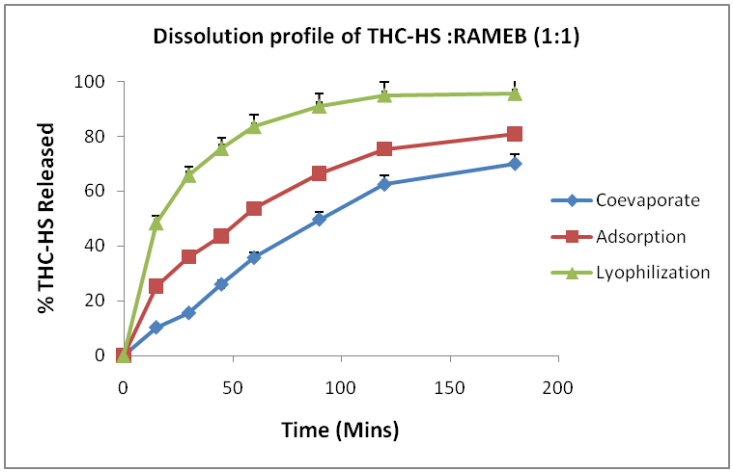

Figure 5-1: Dissolution profiles of THC-HS:RAMEB lyophilized solid dispersions (different methods)

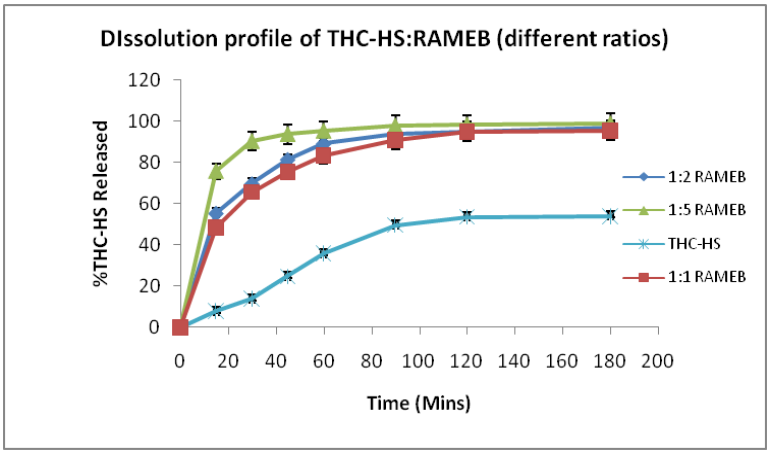

Figure 5-2: Dissolution profiles of THC-HS:RAMEB lyophilized solid dispersions in different ratios

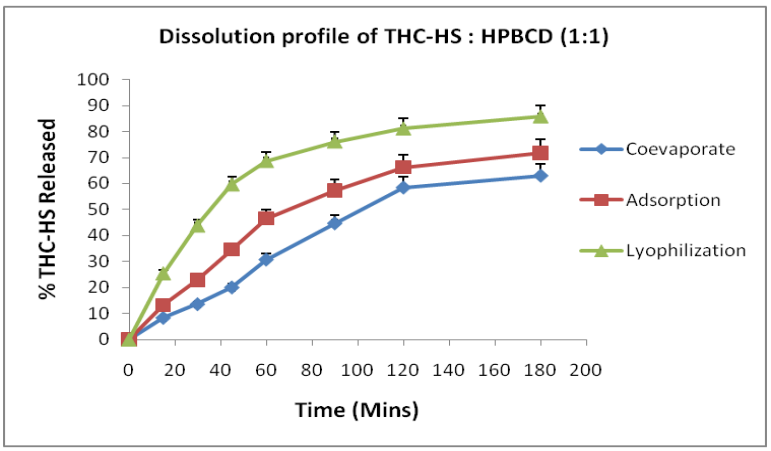

Figure 5-3: Dissolution profiles of THC-HS:HPBCD lyophilized solid dispersions in different methods

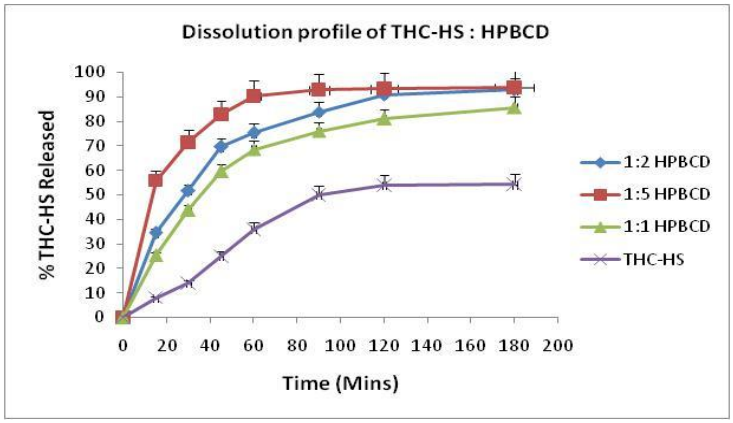

Figure 5-4: Dissolution profiles of THC-HS:HPBCD lyophilized solid dispersions in different ratios

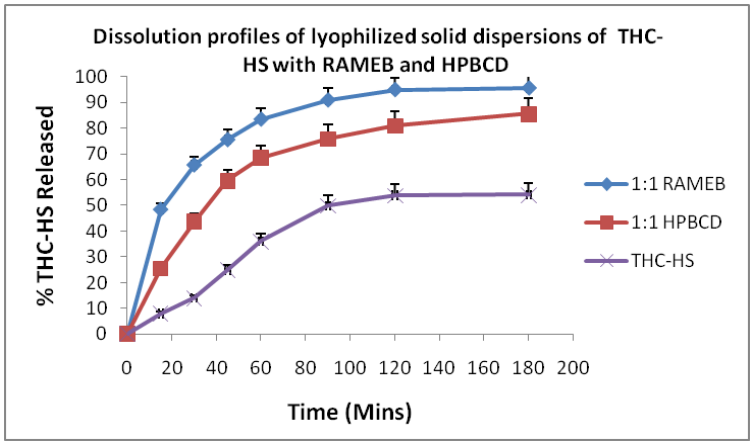

Figure 5-5: Comparison of dissolution profiles of THC-HS:RAMEB & THC-HS:HPBCD lyophilized solid dispersions

5.3.2. Accelerated stability testing of THC-HS:RAMEB solid dispersions

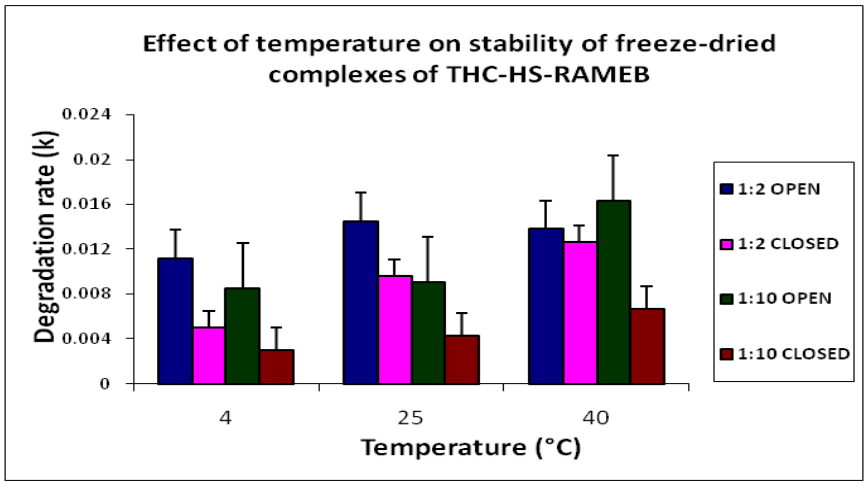

Figure 5-6: Degradation rate constants of lyophilized complexes of THC-HS/RAMEB

The degradation rate of the THC-HS in freeze-dried inclusion complexes with RAMEB decreased with increasing concentration of RAMEB. In open vials, percent drug remaining at the end of 21 days at 25°C, in 1:2 and 1:10 inclusion complexes of THC-HS/RAMEB was 49% and 66%, respectively. In closed vials, percent drug remaining in the 1:2 and 1:10 inclusion complexes of THC-HS/RAMEB was 64% and 83%, respectively at the end of 21 days (fig.5-6; fig.5-7). The maximum degradation was observed when the lyophilized inclusion complex of THC-HS:RAMEB were stored in open containers (fig.5-7). Higher concentrations of RAMEB conferred more stability to the included prodrug.

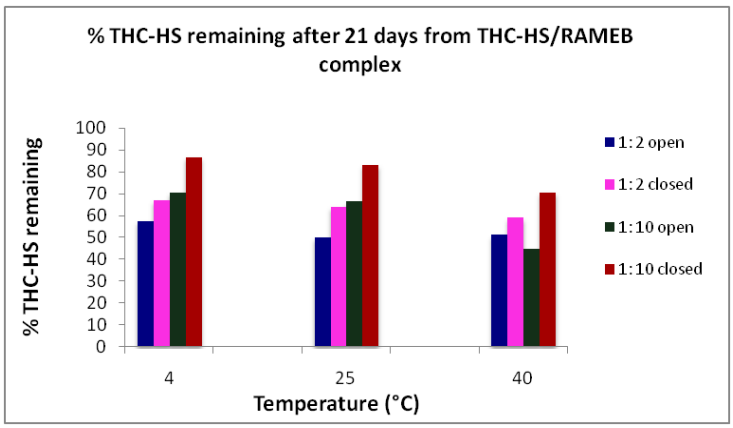

Figure 5-7: Thermal stability of lyophilized complexes of THC-HS/RAMEB

In open conditions, oxidation as well as hydrolysis of THC-HS due to moisture led to higher degradation of the prodrug in the complex. The activation energy was also found to be the highest for the THC-HS:RAMEB (1:10) complex as also indicated by a low degradation constant (Fig.5-6 & Table 5-1).

Table. 5-1: Activation energy of lyophilized complexes of THC-HS/RAMEB

THC-HS :RAMEB complex	**Activation energy, E_a (J/mol)**
1:2 open	0.868
1:10 open	2.706
1:2 closed	3.342
1:10 closed	3.840

Most accelerated testing models are based on the Arrhenius equation:

$$\ln(k) = \frac{-E_a}{R}\frac{1}{T} + \ln(A)$$

The above equation describes the relationship between storage temperature and degradation rate. Use of the Arrhenius equation permits a projection of stability from the degradation rates observed at high temperatures. Activation energy, the independent variable in the equation, is equal to the energy barrier that must be exceeded for the degradation reaction to occur. When the activation energy is known (or assumed), the degradation rate at low temperatures may be projected from those observed at "stress" temperatures.[209]

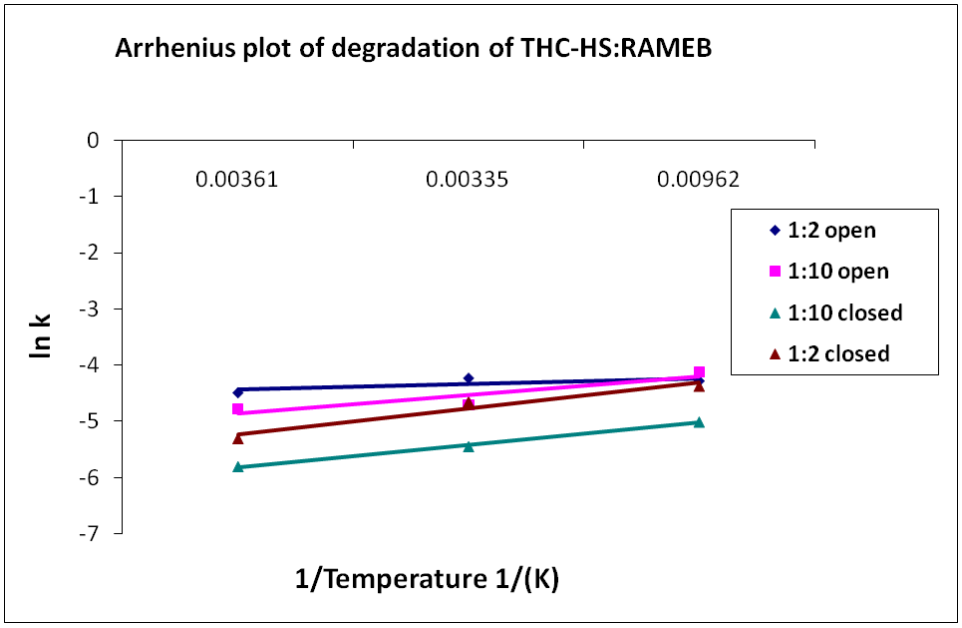

Figure 5-8: Arrhenius plot of lyophilized complexes of THC-HS/RAMEB

The activation energy was highest for THC-HS:RAMEB (1:10 closed) which correlated well with the stabilization effect of this formulation on THC-HS. The degradation rate constants were also the lowest for THC-HS:RAMEB (1:10 closed) system as evident from Fig.5-6.

5.3.3. *In Vitro Permeability* Studies of lyophilized THC-HS:RAMEB solid dispersions

It is generally recognized that cyclodextrins act as true carriers by keeping the hydrophobic drug molecules in solution and deliver them to the surface of the biological membrane, where they partition into the membrane. Cyclodextrins can enhance drug permeation by increasing drug availability and stability at the surface of the biological barriers. However, derivative cyclodextrins, especially methylated cyclodextrins, act as absorption enhancers by different pathways. These hydrophobic cyclodextrins act as absorption enhancers, probably, by transiently changing membrane permeability, overcoming the aqueous diffusion barrier and opening tight junctions. Methyl-b-cyclodextrin, a more hydrophobic cyclodextrin, can permeate the buccal mucosa to form inclusion complexes with hydrophobic molecules, namely lipids from the cellular membrane, interacting strongly with these lipids; it could modify buccal mucosa permeability and could act as penetration enhancer for the buccal route.

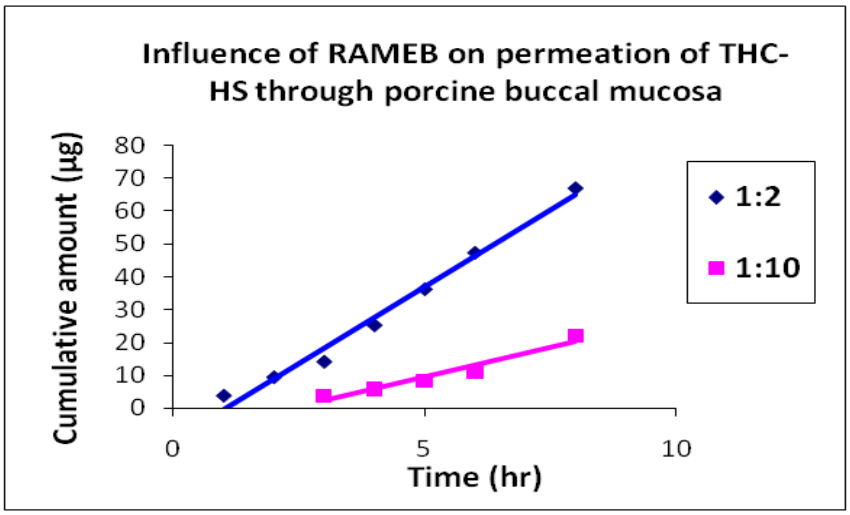

Figure 5-9: *In vitro* permeability profile of THC-HS:RAMEB lyophilized solid dispersions

The permeability of THC-HS/RAMEB (1:2) lyophilized complex was increased four-fold and that of the 1:10 complex increased two-fold compared to the permeability of the THC-HS alone. The lag time associated with the diffusion of THC-HS:RAMEB (1:2) complex was negligible, whereas that of 1:10 inclusion complex of THC-HS:RAMEB was approximately 2 hr (fig..5-9). This can be attributed to the higher bound concentration of THC-HS with increasing concentrations of RAMEB.

Table. 5-2: Permeability parameters for THC-HS:RAMEB lyophilized solid dispersions

Diffusion parameters	**THC-HS:RAMEB (1:2)**	**THC-HS:RAMEB (1:10)**
Flux (μg/cm^2.hr)	9.317	3.5817
Permeability (cm/sec)	5.084 x 10^{-6}	1.955 x 10^{-6}

The stability of THC-HS was increased by its complexation with RAMEB. Lyophilization was found to be the optimum method of processing THC-HS in a film formulation. Freeze-drying also resulted in higher content uniformity than the physically mixed prodrug and RAMEB. Higher concentrations of RAMEB led to lower flux and total amount permeated across the buccal mucosa in permeability profiles. Hence an optimum amount of RAMEB to be added in films will have to be studied and ascertained to achieve greater stability and higher permeation as well across the buccal mucosa.

5.4. Conclusion

Out of the two modified cyclodextrins, RAMEB was better able to solubilize the drug and enhance the rate of release of THC-HS form solid dispersions as compared to HPBCD. The

flux across the buccal mucosa was increased due to the ability of RAMEB to increase the concentration of THC-HS at the interface of the buccal membrane. The lyophilized solid dispersions of THC-HS-RAMEB were more stable than the drug itself.

CHAPTER 6

Formulation studies of lyophilized solid dispersions of

THC-HS-RAMEB

6.1. Introduction

Δ^9 –Tetrahydrocannabinol (THC), the major pharmacologically active constituent of *cannabis sativa* has been utilized for the treatment of nausea and vomiting in cancer chemotherapy. Due to THC itself being highly lipophilic, water-insoluble and unstable drug, an effective dosage form has not developed to date. We have initially reported the formulation development of more hydrophilic prodrugs of THC viz; Δ^9–Tetrahydrocannabinol hemisuccinate (THC-HS) and Δ^9–Tetrahydrocannabinol hemiglutarate (THC-HG).[30-33,172] Though there have been initial reports of formulation development of THC-HS transmucosal films, there is no research elucidating the synergistic effect of cyclodextrins and various other formulation variables such as anti-oxidants, plasticizers and pH modulating agents on stabilization of THC-HS in polymeric transmucosal films

The purpose of the research work was to enhance the stability of lyophilized solid dispersions of THC-HS:RAMEB in polymeric transmucosal film system.

6.2. Methods

6.2.1. Feasibility testing for preparation of hot-melt cast films of lyophilized THC-HS-RAMEB solid dispersions

The lyophilized powders of THC-HS/RAMEB (1:1) were physically mixed with polyoxyethylene oxide (PEO; PolyOx®) N-80 and exposed to hot-melt processing temperatures of 115-125°C, for 5 minutes, to produce a polymeric patch.

Three types of formulation methods were adopted to assess the stability of THC-HS in presence of RAMEB in a hot-melt cast film.

F1 – THC-HS:RAMEB (1:1) lyophilized inclusion complex + PEO-N80

F2 – THC-HS + RAMEB + PEO-N80 (Physical mixture)

F3 – THC-HS + PEO-N80 (Physical mixture)

Content uniformity of each type of formulation was determined to establish the most feasible method of incorporation of lyophilized THC-HS:RAMEB solid dispersions in hot-melt cast patches.

6.2.2. Effect of RAMEB on stability of THC-HS in hot-melt cast polymeric films

Hot-melt cast films of THC-HS:RAMEB with PEO-N80 were prepared to determine effect of RAMEB on the post-processing content of THC-HS and its storage stability. THC-HS was lyophilized with RAMEB (1:1) at -50°C in a lyophilizer. The lyophilized powders of THC-HS/RAMEB (1:1) were physically mixed with polyoxyethylene oxide (PEO; PolyOx®) N-80 and exposed to hot-melt processing temperatures of 115-125°C, for 5 minutes, to produce a polymeric patch. The patches were stored at four different temperatures viz; -20°C, 4°C, 25°C and 40°C.

6.2.3. Effect of Plasticizers on stability of THC-HS in hot-melt cast polymeric films

Screening of Plasticizers

PEO softens at approximately 80° C; however, the nature of the polymers necessitates the use of a processing aid in order to produce a uniform melt. For this purpose, various potential plasticizers were tested to evaluate miscibility with PEO. These include vitamin E succinate (VES), acetyl tributyl citrate (ATBC), PEG 8000, and triacetin. The suitable

plasticizers/additives were further used to facilitate the film formation when incorporated with the drug for studying the stability of THC-HS in presence of RAMEB.

The patches containing the suitable plasticizers selected from the screening studies were further stored at four different temperatures, as mentioned above, for stability purposes. The types of plasticizers with their concentration used are given below:

Triacetin (5%w/w), PEG-8000(5%w/w), VES (5%w/w), ATBC (5%w/w), RAMEB (1%w/w)

6.2.4. Effect of pH modulation on stability of THC-HS in hot-melt cast polymeric films

PEO matrices incorporated with various pH modifiers along with THC-HS:RAMEB (1:1) were fabricated utilizing a hot-melt method at 115°C-125°C. The various pH modifiers studied included citric acid, fumaric acid, malic acid, and sodium deoxycholate. The processed matrices with selected pH modifiers were stored at four different temperatures -20°C, 4°C, 25°C and 40°C and analyzed for THC-HS and THC content at various time intervals for up to 3 months utilizing the HPLC method.

6.2.5. Effect of anti-oxidants on stability of THC-HS in hot-melt cast polymeric films

Earlier preformulation studies have revealed that THC-HS is prone to hydrolysis and oxidation. THC-HS is hydrolyzed to its parent compound, THC which might be oxidized to cannabinol (CBN) (oxidative degradation product of THC) peak in HPLC analysis. Hence the effect of various classes of anti-oxidants on the stability of THC-HS was investigated.

PEO-N80 polymeric matrices were fabricated incorporated with various anti-oxidants, along with the lyophilized solid dispersions of THC-HS:RAMEB (1:1) utilizing the previously

described hot-melt method. The processed matrices were checked for the post-processing content of THC-HS and THC utilizing the HPLC method. Two classes of antioxidants were utilized:(i) free radical scavengers (BHT, BHA, propyl gallate), (ii) reducing agents or oxygen scavengers (ascorbic acid). A combination of these antioxidants was also utilized.

6.3. Results and Discussion

For oral transmucosal delivery, a flexible polymeric matrix system that adheres to the mucosa for a predetermined period of time is desirable. Hot-melt processing has been demonstrated to be a viable method for the preparation of drug –incorporated polymeric films. Hot-melt processing requires a pharmaceutical grade polymer that can be processed at relatively low temperatures (low melting point or glass transition temperatures) due to thermal sensitivity of many drugs. In the present study, lyophilized solid dispersions of THC-HS and RAMEB were incorporated in to the hot-melt cast polymeric matrices of Polyox™ N-80 (PEO N-80) intended for transmucosal delivery of THC-HS through the buccal mucosa. For fabrication of hot-melt polymeric matrices, a processing temperature of at least 20-30°C above the melting point of semi-crystalline polymer or glass transition temperature of an amorphous polymer is desirable to lower the polymer melt viscosity. The low melting temperature (melting range 57-73°C, based on molecular weight) of PEO-N80 facilitates thermal processing to be performed at 110-120°C. Hence PEO N-80 was chosen as the base polymer for fabricating drug incorporated matrices.

6.3.1. Effect of processing temperature on stability of THC-HS:RAMEB complex

Formulation type legends

F1 –THC-HS:RAMEB (1:1) freeze-dried inclusion complex + PEO-N80

F2 – THC-HS + RAMEB + PEO-N80 (Physical mixture)

F3 – THC-HS + PEO-N80 (Physical mixture)

The effect of processing temperature was profound in patches containing only PE0 N-80 and THC-HS wherein the percent drug remaining was only 13%. The patches containing the lyophilized complex of THC-HS/RAMEB showed significantly less degradation at the same conditions wherein the percent drug remaining was 49%. THC-HS is degraded to THC and CBN as determined by HPLC analysis in all the samples. It is well-known that mono- and polyetheric compounds like polyoxyethylene oxides, easily undergo free radical autooxidation during storage and upon exposure to heat, light and transition metals to form peroxides which can be a source of drug degradation by oxidation.[146] Also, PEO N-80 consists of ether and hydroxyl groups which are capable of potentiating the hydrolysis of THC-HS. RAMEB, however is observed to shrink the hydrolysis of THC-HS to THC to a considerable extent as noted form Figure 6-1. This is due to the fact that RAMEB protects THC-HS molecule from hydrolysis by encapsulating its hydrophobic aromatic ring system in its cavity as well as forming hydrogen bonds with the hemisuccinate ester linkage.

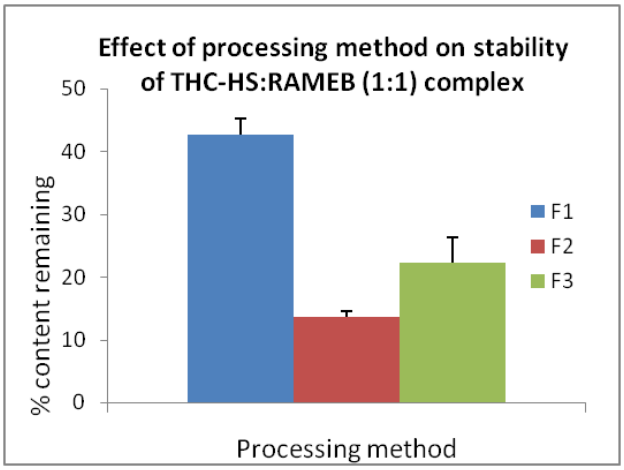

Figure 6-1: Effect of processing method on stability of THC-HS:RAMEB

6.3.2. Content uniformity of hot-melt cast films containing THC-HS:RAMEB complex

The content uniformity of freeze-dried lyophilized powders of THC-HS:RAMEB complex as well as physical mixtures of THC-HS and RAMEB was determined to optimize the method of incorporation of RAMEB in the films.

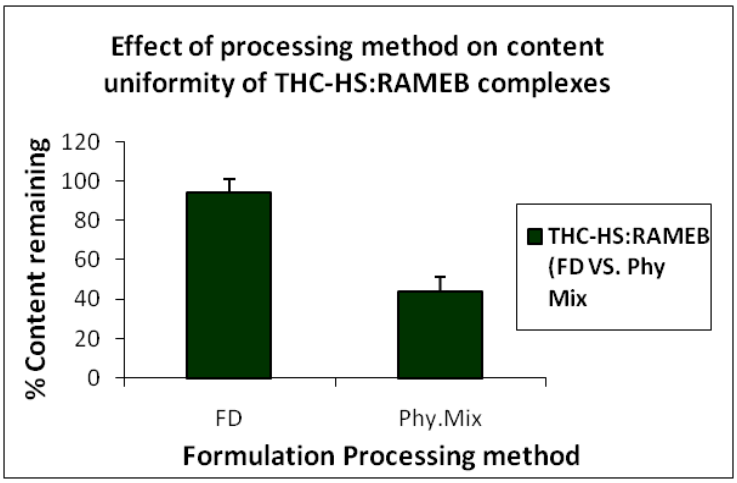

Figure 6-2: Effect of processing method on content uniformity of THC-HS:RAMEB complexes

Lyophilized powders of THC-HS and RAMEB exhibited a satisfactory content uniformity and the handling of powders was easier than the plain physical mixtures of THC-HS:RAMEB (fig 6-2). Lyophilization led to more intimate mixing and ease in complex formation of THC-HS with RAMEB.

6.3.3. Effect of RAMEB on stability of THC-HS in hot-melt cast polymeric matrix

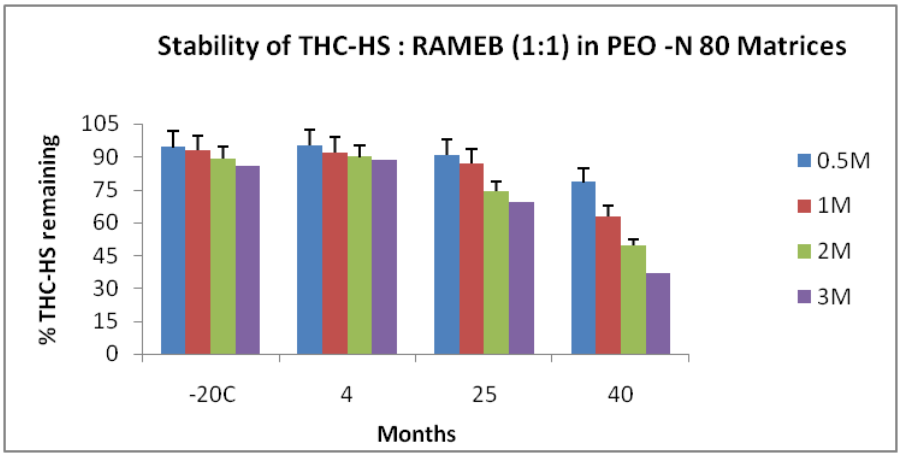

Figure 6-3: Stability of THC-HS:RAMEB (1:1) in PEO-N80 Matrices

THC-HS:RAMEB lyophilized solid dispersions incorporated in a polymeric hot-melt cast patch were stable at -20°C and 4°C up to 2months but showed significant degradation at 25°C and 40°C (fig 6-3).

6.3.4. Effect of Plasticizers / Processing aids of THC-HS in hot-melt cast polymeric matrix

Plasticizers are typically low molecular weight compounds capable of softening polymers to make them more flexible. The use of polymeric carriers in hot-melt extrusion often requires the incorporation of a plasticizer into the formulation to improve the processing conditions

during the manufacturing of the extruded dosage form or to improve the physical and mechanical properties of the final product.

Plasticization of the polymer is generally attributed to the intermolecular secondary valence forces between the plasticizer and the polymer. Plasticizers are able to decrease the glass transition temperature and the melt viscosity of a polymer by increasing the free volume between polymer chains. In so doing, the ease of movement of polymer chains[150] with respect to each other is dramatically reduced. Plasticizers were also found to facilitate the fusion process of semi-crystalline polymers. With the addition of a plasticizer, a hot-melt extrusion process can be conducted at lower temperatures and with less torque. Both the active ingredient and the polymer will be more stable during the extrusion process due to these improved processing conditions. Various plasticizers such as triacetin, PEG 8000, vitamin E succinate (VES), acetyl tributyl citrate were incorporated in to PEO N80-THC-HS-RAMEB matrices.

Overall, incorporation of plasticizers lowered the degradation rate of THC-HS in THC-HS:RAMEB solid dispersions in hot-melt cast films as compared to PEO-N80 matrices only (fig 6-4). Also, the films processed in presence of plasticizers were easy to fabricate and showed excellent content uniformity. The plasticizers acted synergistically along with RAMEB to reduce the degradation of THC-HS in the hot-melt cast films.

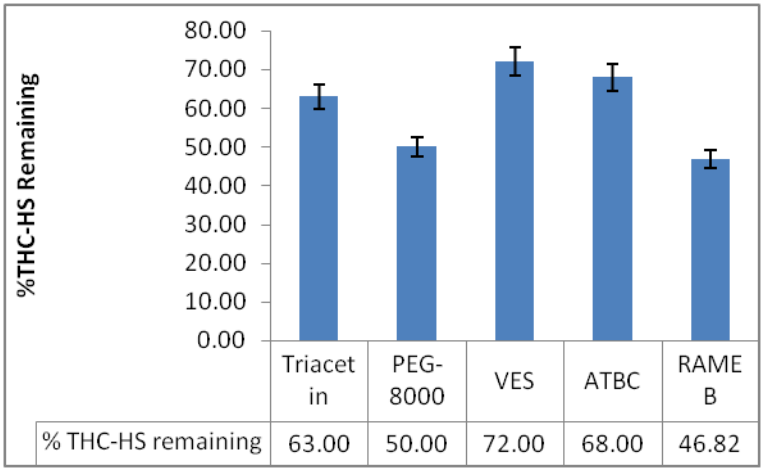

Figure 6-4: Effect of plasticizers on polymeric matrices of THC-HS:RAMEB

VES was found to be the best plasticizer among all the plasticizers tested followed by ATBC as regards to the post-processing content of THC-HS. The degradation of drug in the various PEO matrices was found to be in the order of PE) N80 only > PEO N80-RAMEB > PEO N80-RAMEB- PEG 8000 > PEO N80-RAMEB-triacetin > PEO N80-RAMEB- ATBC > PEO N80-RAMEB- VES. It is observed that THC-HS degraded to a higher extent in the presence of hydrophilic plasticizers like PEG 8000, triacetin as compared to relatively hydrophobic plasticizers such as VES and ATBC. These findings are consistent with Munjal *et al* and Thumma *et a*[30,172,210]*l* who also reported significant degradation of THC prodrugs in presence of hydrophilic excipients.

The stability testing provides evidence on how the quality of an active varies with time under the influence of a variety of environmental factors such as temperature and humidity. The stability testing results indicate that though the immediate post-processing content of THC-HS was improved with the addition of plasticizers in hot-melt transmucosal films, the content of THC-HS at the end of three months dropped significantly at all temperature and humidity conditions (fig 6-5). These results suggest that further optimization of the formula is required to

improve the post-processing content of THC-HS in transmucosal films. In stability testing, overall, plasticizers were found to work synergistically with RAMEB to augment the stabilization effect of RAMEB in lyophilized solid dispersions of THC-HS.

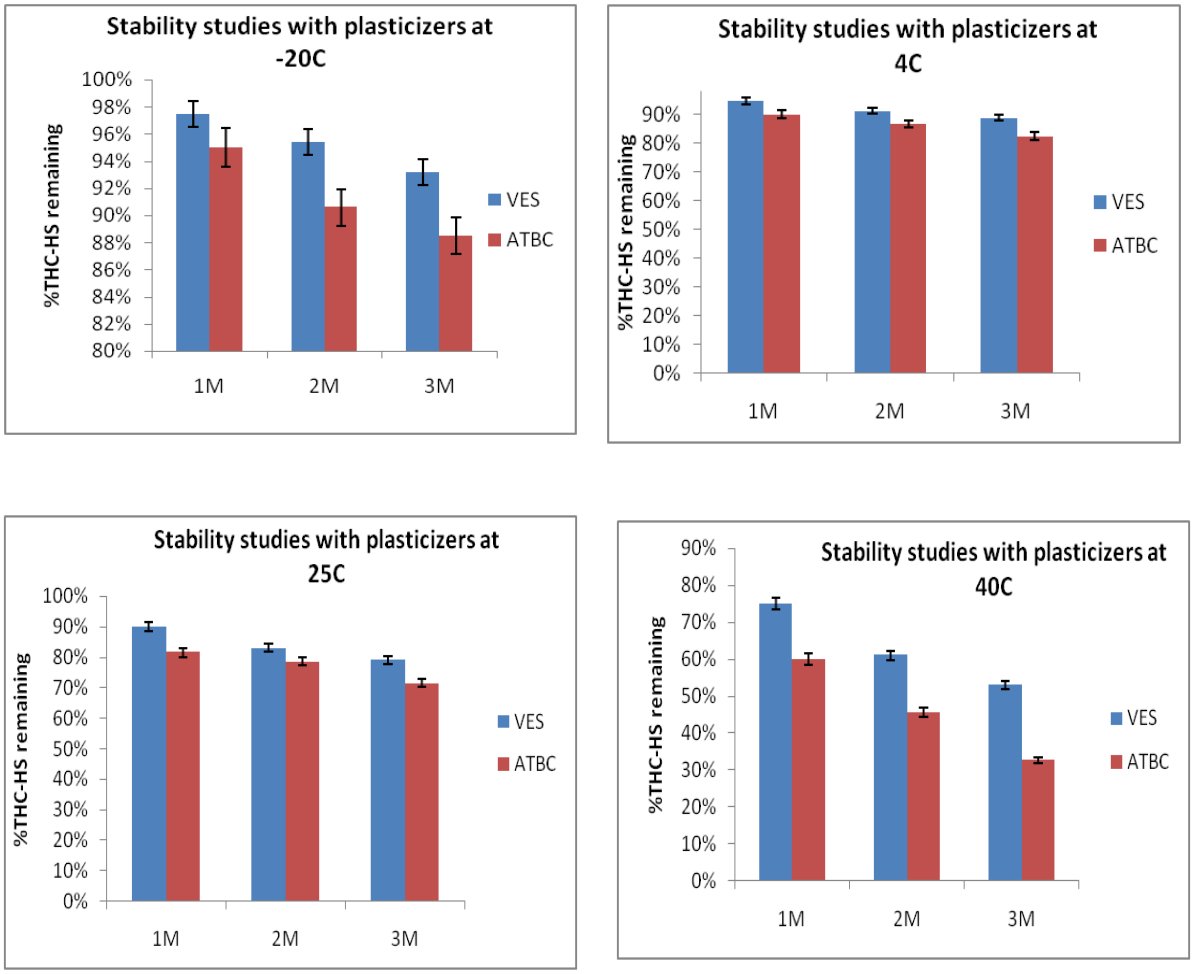

Figure 6-5: Stability testing of plasticizers in polymeric matrices of THC- HS:RAMEB

6.3.5. Effect of pH modulation of THC-HS in hot-melt cast polymeric matrix

It is well-known that the degradation arte of drugs is affected by the microenvironmental pH to which they are exposed in the solid dosage forms. Studies on enhancing the stability of drugs with the use of pH modulators into the formulations have been reported previously.[211,212]

THC-HS has been reported to exhibit alkali-catalyzed hydrolysis and it might be beneficial to incorporate acidic pH modulating agents to improve the hydrolytic stability of THC-HS in hot-melt transmucosal films. Various acid pH modulating agents such as citric acid, malic acid, fumaric acid were incorporated into PEO N80-THC-HS-RAMEB matrices. It was found that citric acid had the most profound positive effect on stability of THC-HS in transmucosal films.

Table.6-1: pH modulation achieved by different pH modulators

Patch formulation	%w/w	pH	% THC-HS remaining
Citric acid	2%	4	82 ±0.97
Malic acid	2%	4.78	75 ± 1.2
Fumaric acid	2%	5.32	69 ± 0.73
Sodium Deoxycholate (NaDC)	2%	7.6	57 ± 1.48
Citric acid + 2% NaDC	2%	4.56	72 ± 1.37

THC-HS:RAMEB polymeric matrices of PEO-N80 containing citric acid reduced the degradation of THC-HS considerably with 82% post-processing content as against 46 % in patches containing no acid modulating agents (fig 6-6). The other acid modifiers did not seem to offer any advantage over citric acid. The results of stability testing also indicated a significant improvement in the content of THC-HS in transmucosal films.

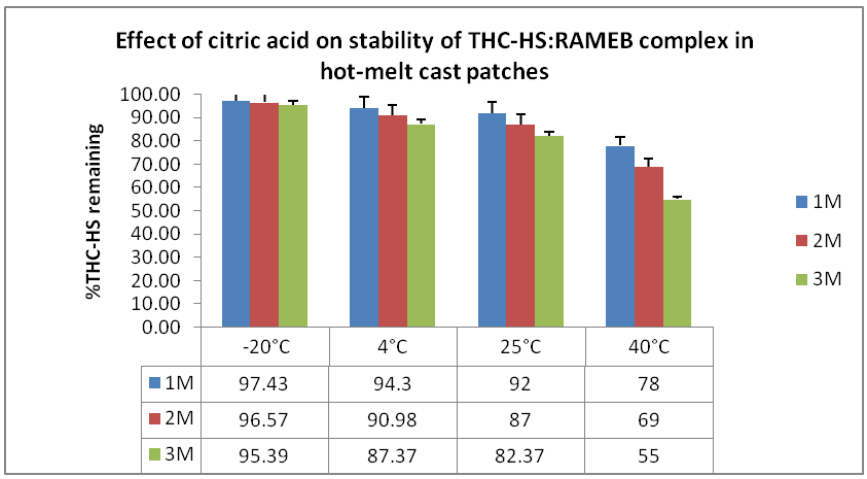

Figure 6-6: Stability testing of citric acid as pH modifier in polymeric matrices of THC-HS:RAMEB

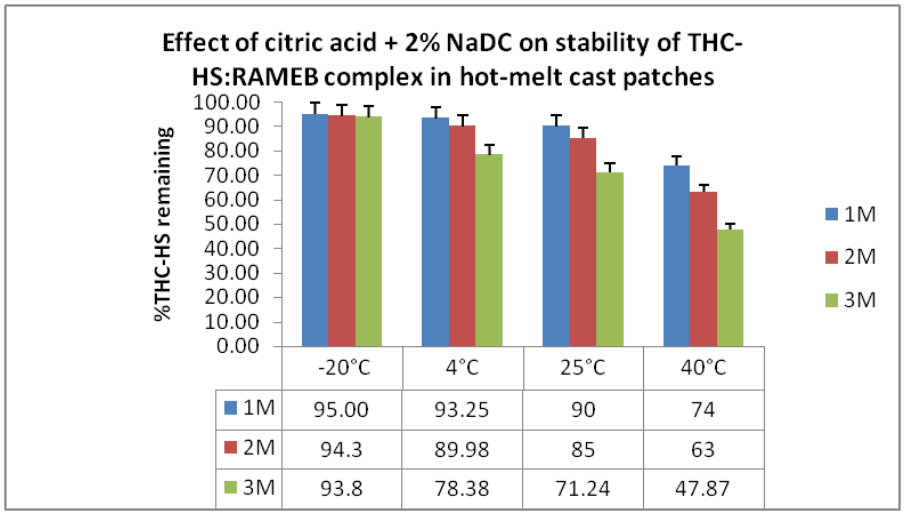

Figure 6-7: Stability testing of citric acid + Sodium deoxycholate as pH modifier in polymeric matrices of THC-HS:RAMEB

The effective pH range for stable hot-melt cast PEO N80 matrices of THC-HS :RAMEB solid dispersions were found to be in the range of 4.0-4.5.

6.3.6. Effect of anti-oxidants of THC-HS in hot-melt cast polymeric matrix

Drugs may undergo oxidation due to reactive impurities that are added during polymer manufacturing or generated upon exposure of the excipient or formulation to light, heat or

metals.[146] THC formed after the hydrolysis of THC-HS is known to degrade by oxidation in addition to hydrolysis of the ester bond in prodrug. Polyethylene oxide has been reported to be protected from free radical and oxidative degradation by the incorporation of an antioxidant. Various classes of antioxidants were incorporated into the PEO N80-THC-HS-RAMEB matrices and stored at different temperature and humidity conditions to assess their role in preventing the prodrug degradation. The concentrations of antioxidants used in the study are given in table 6-2.

Table.6-2: Screening of anti-oxidants

Antioxidant	% w/w
PEO-N80	-
RAMEB	1
VES	5
Citric acid	2
BHT	0.02
BHA	0.2
Propyl gallate	0.5
Ascorbic acid	1
BHT + Propyl gallate	0.02 + 0.5
BHT + Ascorbic acid	0.02 + 0.5

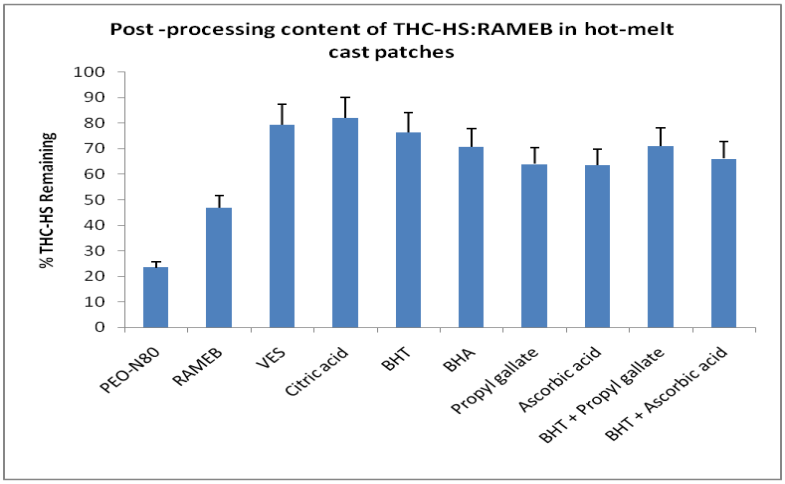

Figure 6-8: Effect of antioxidants on stability of THC-HS

Almost all of the antioxidants incorporated into the PEO-N80-THC-HS:RAMEB matrices reduced the degradation of THC-HS as compared to PEO-N80-THC-HS:RAMEB only matrices. However, degradation of the drug continued even in the presence of anti-oxidants.

BHT and the combination of BHT + Propyl gallate showed significant advantage ($p < 0.05$) with 76.35% and 71% post-processing content over the other anti-oxidants (Fig.6-8).

6.4. Conclusion

The results of the present study clearly underlines the importance of formulation variable sin producing stable polymeric transmucosal films of THC-HS. Further optimization of the formula needs to be done to study the critical formulation variables for stability of THC-HS in hot-melt transmucosal films as well as the interactions, if any between the individual excipients.

CHAPTER 7

Application of factorial design to identify critical formulation variables in the design of hot-melt transmucosal films of THC-HS

7.1. Introduction

Δ^9-Tetrahydrocannabinol (THC), the major pharmacologically active constituent of *Cannabis sativa* exhibits therapeutic potential in the treatment of nausea and vomiting during cancer chemotherapy. The only commercially available USFDA approved dosage form for treatment in chemotherapy-related nausea and vomiting is the soft gelatin capsule for oral administration, marketed in the USA as Marinol® and two of its generic versions. In this formulation, however, the drug has limited stability and therefore has to be stored at low temperatures (4 °C). Moreover, the oral bioavailability of the drug is low ($\sim$ 6%) and inconsistent, which is mainly due to its high first-pass metabolism and poor solubility.[163] In addition to the pharmacokinetic limitations, the physicochemical properties of THC present a major challenge in the development of a suitable dosage form. THC is a poorly water-soluble, amorphous substance which is sticky, resin-like and highly viscous, making it difficult to handle and process. Furthermore, the instability of THC, especially in acidic solutions, and when exposed to heat, air and light has been reported by various researchers. To overcome the challenges of THC molecules in terms of its unfavorable physicochemical properties as well as high first pass metabolism, we have designed novel hydrophilic prodrugs of THC to be administered by buccal route in the form of transmucosal films. To enhance the solubility and stability of a novel first-generation THC prodrug, Δ^9-Tetrahydrocannabinol hemisuccinate (THC-HS), various formulation additives such as random methylated beta cyclodextrin (RAMEB as solubilizer), Vitamin E succinate (VES as a plasticizer) and citric acid (CA as a pH modulating agent) were employed to produce a stable hot-melt cast transmucosal films for buccal application.

During formulation development of a stable product, design of experiments methodology allows the systematic evaluation of the effect of formulation variables, with a minimum number of experiments, in order to identify the critical parameters.[213]

Quality by design (QbD) is one such FDA initiative to pharmaceutical development (FDA guidance of industry, 2006). The objective of QbD approach is to design a process in such a way that manufactures pharmaceuticals that consistently meet critical quality attributes. A process and formulation can be better understood if the design is based on multivariate analysis which identifies critical formulation factors and as well as root causes of variability in the formulation. In the current study, we studied the effects of the formulation additives on the post-processing content as well as the release rate of THC-HS from the transmucosal films using statistical 2^3 full factorial design.

7.2. Materials

The following chemicals were used as received: randomly methylated beta-CD (RAMEB) were purchased from Sigma Aldrich with a degree of substitution of 1.7 and 0.6, respectively. PEO [PolyOx® WSR N-80 (PEO N-80), MW 200,000 Da] were kindly donated by Dow Chemical Company (Midland, MI). Vitamin E succinate (VES), citric acid anhydrous, malic acid, fumaric acid, sodium lauryl sulfate (SLS) were purchased from Spectrum Chemical, Inc. (Gardena, CA). HPLC-grade water was freshly prepared in the laboratory (by Nanopure systems, Barnstead, Dubuque, IA). HPLC-grade acetonitrile and methanol and were obtained from Fisher Scientific, Fair Lawn, NJ; THC-HS (in hexane) and THC (in absolute ethanol) were provided by ElSohly Laboratories Inc, Oxford, MS.

7.3. Methods

7.3.1. Experimental Design

A 2^3 full factorial design was created to determine and optimize the effect of the three formulation factors using two responses. Three continuous factors, percent content of RAMEB, VES and CA were tested at two levels designated as -1 and +1, respectively with 0 as the centerpoint. The dependent variables analyzed were as follows: post-processing content of THC-HS and dissolution profile of THC-HS at 60 minutes. Table.1 lists the factor levels for 11 film formulations as per the design.

7.3.2. Preparation of hot-melt cast polymeric films of THC-HS

Hot-melt cast films of lyophilized solid dispersions of THC-HS:RAMEB with PEO-N80 were prepared to determine the effect of RAMEB, VES and CA on the post-processing content and the release rate of THC-HS from its polymeric matrices. THC-HS was lyophilized with RAMEB at -50°C in a lyophilizer. The lyophilized powders of THC-HS/RAMEB were physically mixed with polyoxyethylene oxide (PEO; PolyOx®) N-80 and exposed to hot-melt processing temperatures of 110°C, for 5 minutes, to produce a polymeric film. Briefly, a die containing a 13-mm diameter opening was placed on top of a brass sheet and heated at 110 °C. Approximately 200 mg of the physical mixture of lyophilized solid dispersions of THC-HS and RAMEB, PEO N80 and other excipients was positioned in the orifice of the die, and compressed using a punch. This compressed mixture was heated for 5 min to form a melt, followed by cooling under room conditions to form a thin polymeric film. Film thickness ranged from 1.1 to 1.3 mm. The diameter of the films produced was approximately 12.9 ± 0.2 mm.

7.3.3. *In vitro* characterization

Sample preparation for post-processing content of THC-HS from transmucosal films

A weighed portion of the THC-HS or drug-incorporated polymeric matrix was dissolved in a known volume of methanol by sonicating it for 10 min. The resulting solution was centrifuged at 16000 rpm for 10 minutes, supernatant transferred into vials and 20 μL was injected into the HPLC column for drug analysis.

7.3.4. *In vitro* release studies

The effect of all the formulation additives on the release of THC-HS from the PEO matrices was investigated. All the selected excipients were incorporated into the PEO matrices along with the lyophilized solid dispersions of THC-HS and RAMEB utilizing the hot-melt method at 110º C. *In vitro* release studies (n = 3) were performed on these matrices utilizing a Hanson SR8-Plus dissolution test system according to USP 31 apparatus 5, paddle over disk method. The polymeric matrix was sandwiched between the watch glass and a mesh so that the release was unidirectional. Five hundred milliliters of pH 6.8 phosphate buffer containing 0.5% w/v SLS at 37 °C was used as the dissolution medium and the paddle rotation speed was 50 rpm. Samples were collected at pre-determined time intervals and replaced with an equal volume of the fresh dissolution medium. The samples were then centrifuged at 16000rm for 15 minutes and supernatant analyzed by a validated HPLC method. The cumulative percent of drug dissolved at the end of 60 minutes (T_{60} %) was used as the endpoint for calculations in the factorial design.

7.3.5. Chromatographic analysis

The chromatographic system consisted of a Waters 600 pump and a dual wavelength Waters 2487 UV detector (Waters Corp., Milford, MA). A Symmetry 5µ C-18, 250 mm × 4.60 mm column (Waters Inc.), was used for the detection of the drug. The mobile phase consisted of 80%acetonitrile and 20% pH 3 monobasic potassium phosphate buffer containing 0.1% triethyl amine, pH adjusted to 3 with phosphoric acid. The flow rate was maintained at 1.2 mL/min, with THC-HS and THC eluting within 15 min. The injection volume was 20 µL, and the column effluent was monitored by UV absorption at 215 nm. The temperature of the column was maintained at 25 °C.

7.3.6. Statistical Analysis

StatGraphics™ Centurion (StatPoint, Inc., Version XV) was used to generate the 2^3 full factorial study designs and to perform the statistical analysis. Data obtained from the experimental formulation testing was analyzed by analysis of variance (ANOVA). The polynomial equation of the model is as follows:

$$Y = b_0 + b_1 A + b_2 B + b_3 C + b_{12} AB + b_{13} AC + b_{23} BC$$

where Y is the dependent variable; b_0 is the intercept; b_1 to b_{23} are the regression coefficients; and A, B and C are the independent variables selected for the experiments.

7.4. Results and Discussion

Eleven formulations were prepared (Table 1 & 2), as designed by the full factorial experimental design for the three factors studied. Table 3 gives the measured post-processing content and the release of THC-HS from the transmucosal films at the end of 60 minutes. The

design was evaluated by multiple linear regression analysis, and the mathematical relationships in the form of regression equations for the measured dependent variables along with the other statistical parameters are given in Table 4.

The results clearly indicate that the content as well as the release of THC-HS from the films is strongly affected by the variables selected for the study. This is also reflected by the wide range of values for coefficients of the terms mentioned in the equations in Table 4. The main effects of A, B and C represent the average result of changing one variable at a time from its low level to its high level. The interaction terms (AB, AC, BC) show how the dependent variables change when two independent variables are simultaneously changed. The negative coefficients in the equation represents an inverse relationship between a response and factor where as a positive value represents a favorable response.

Table 7-1. Factor Levels for the 2^3 Factorial Design

Coded levels of Independent variables	**-1**	**1**	**0 (centerpoint)**
Factor A : CA (%)	0.25	2	1.125
Factor B: VES (%)	0.5	5	2.75
Fcator C: RAMEB (%)	4	8	6
Dependent variables	Post processing content of THC-HS (Y_1)	% Release of THC-HS at 60 minutes (T_{60} %) (Y_2)	

Table 7-2: 2^3 Factorial Design for independent variables

Independent Variables	**F1**	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**	**F8**	**F9**	**F10**	**F11**
VES (%)	0.5 (-1)	5 (+1)	0.5 (-1)	5 (+1)	0.5 (-1)	5 (+1)	0.5 (+1)	5 (+1)	2.75 (0)	2.75 (0)	2.75 (0)
CA (%)	0.25 (-1)	0.25 (-1)	2 (+1)	2 (+1)	0.25 (-1)	0.25 (-1)	2 (+1)	2 (+1)	1.125 (0)	1.125 (0)	1.125 (0)
RAMEB (%)	4 (-1)	4 (-1)	4 (-1)	4 (-1)	8 (+1)	8 (+1)	8 (+1)	8 (+1)	6 (0)	6 (0)	6 (0)

Table 7-3: Measured Responses (dependent variables) for the film formulations

FORMULATION	**THC-HS CONTENT (%) (n=3) (Y_1)**	**THC-HS RELEASE (T_{60} %)* (n=3) (Y_2)**
F1	60.53±1.28	18.34±2.3
F2	69.58±0.83	16.31±1.97
F3	84.55±0.47	51.76±2.8
F4	86.43±0.32	43.56±3.8
F5	77.56±1.1	38.34±1.45
F6	69.38±0.92	45.63±3.7
F7	95.06±0.61	72.23±3.6
F8	98.57±0.75	61.05±4.2
F9	83.24±1.34	50.32±3.9
F10	85.45±0.94	56.71±2.2
F11	84.67±1.02	58.95 ±3.1

***T_{60} %, % Drug release at the end of 60 minutes**

Table 7-4: Statistical analysis of dependent variables Y_1 and Y_2 along with the regression equations

		% Content – THCHS (Y_1)			% Release-THCHS (Y_2)	
	Coefficient	**F ratio**	**p- value**	**Coefficient**	**F ratio**	**p- value**
Constant(b_0)	48.0604			-9.0049		
CA % (A)	9.24764	44.56	0.0026	24.882	16.38	0.0155
VES % (B)	2.602	0.22	0.6618	-0.08111	0.27	0.6308
RAMEB % (C)	3.195	9.08	0.0394	5.883	10.32	0.0325
R^2		93.2855			87.5061	

7.4.1. Influence of formulation variables on post-processing content of THC-HS in transmucosal films (Y_1)

The post-processing content of THC-HS in transmucosal films is dependent on several process-parameters as well as formulation excipients present in the films. The films were produced using hot-melt cast method wherein THC-HS is exposed to higher temperature (110°C) albeit, for few minutes during which the polymer PEO N80 melts to form a uniform film. For the purposes of this experimental design, the time and the temperature of the melt-casting process were kept constant at 110°C for 5 minutes so as to avoid their influence on the content of THC-HS in the processed films. THC-HS, as discussed earlier [197] is an ester prodrug with a high hydrolytic potential. It is also unstable to heat and oxidation. Hence to improve the stability of THC-HS in hot-melt cast transmucosal films, a proper choice of excipients including plasticizers, pH modulating agents and solubilizers is warranted. Here we have chosen three formulation additives viz., citric acid (pH modulating agent), Vitamin E succinate (VES, plasticizer) and a

modified cyclodextrin, random methylated beta cyclodextrin (RAMEB, solubilizer) to produce an improved formulation of THC-HS. The rapid hydrolysis of ester prodrug, THC-HS to its highly lipophilic and water-insoluble parent drug THC in neutral as well as alkaline pHs results in lower post-processing content of THC-HS in transmucosal films.

The degradation rate and profile of THC-HS are affected by the microenvironmental pH of the polymeric transmucosal films. For overcoming pH-dependent behavior of drugs, pH-modifying excipients (which alter the microenvironment pH inside the formulation) are most commonly used.

This strategy provides an opportunity to improve the stability of a formulation by providing the optimal 'pH' for the drug to maximize its stability. The 'pH' of a formulation is determined by the nature and possibly the concentration of the active ingredient and the various excipients. Acidic or basic ingredients (pH modifiers) can be intentionally added to a formulation for the sole purpose of modifying the 'pH.' Here, we have applied the concept of modifying the microenvironmental pH of THC-HS for enhancing the stability of THC-HS in transmucosal films.

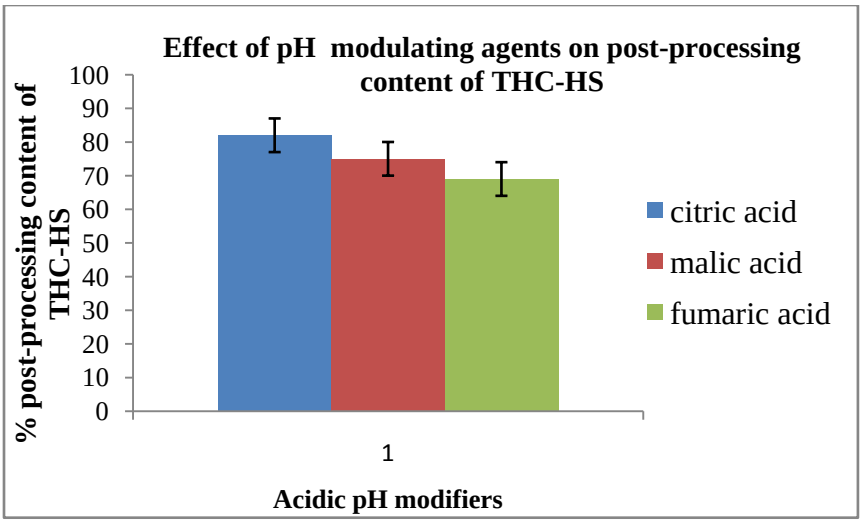

Figure 7-1: Effect of acidic pH modulating agents on post-processing content of THC-HS

It has been shown earlier that THC-HS is stable at acidic pHs between 3.5 -5.5. As a part of preliminary investigation, we studied the effect of different acidic pH modifiers such as citric acid, fumaric acid and malic acid on the post-processing content of THC-HS (Fig.1). Out of all the three pH modifiers, citric acid showed the highest post-processing content of THC-HS. Despite having similar pKa values (˷3), citric acid showed significant enhancement in stability of THC-HS. Due to the higher solubility of citric acid, its saturated solution pH was found to be 4 compared to 5 and 5.32 for malic and fumaric acid respectively.[214] Citric acid was therefore chosen as a pH modulating agent for the optimization process. The appropriate concentration of the pH modifier in a solid formulation required to achieve desired 'pH' control is not always a straightforward decision. A minimum concentration of the pH modifier is probably needed to achieve needed 'pH' control. This concentration is likely dependent on particle size of the formulation ingredients and the pH modifiers and on the manufacturing process. Increasing the pH modifier content above this minimum concentration is not expected to achieve a more effective 'pH' control and hence provides no further stability improvement.

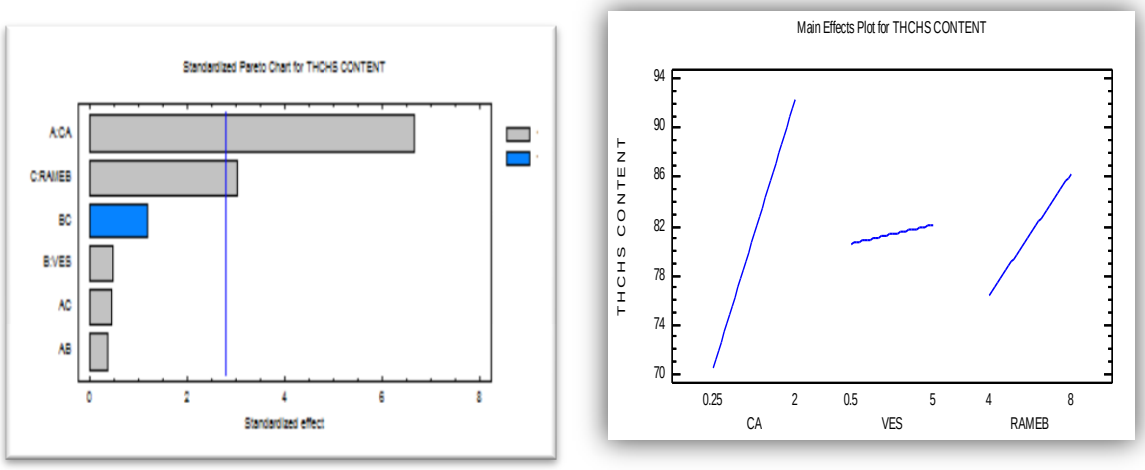

Figure 7-2: Standardized pareto chart and Main effects

for post-processing content of THC-HS (Y_1)

In this case, we have chosen two levels of citric acid, 0.5% and 2% yielding pHs 6.0 and 4.0 respectively. The standardized pareto and the Main Effects plot in Figure.1 illustrate citric acid had a positive effect on content of THC-HS from the film. Citric acid at the highest level in this design reduced the ester hydrolysis of THC-HS to THC thereby increasing its post-processing content. As expected, increasing concentration of citric acid in the formulation increased the post-processing content of THC-HS by the virtue of reducing the microenvironmental pH in the transmucosal film. Increasing the pH of the film formulation beyond pH 4.0 would have caused irritation to the buccal mucosa. Therefore within the range chosen, the confidence that the model can predict the observed value better than the mean was 83.21% and good correlation was obtained between observed and predicted value as indicated by R^2 value of 0.932 (Table 4). From the *F* ratios given for Y_1, it can be concluded that citric acid had the most significant effect ($P < 0.05$) on the post-processing content of THC-HS from the film.

Another factor that significantly impacted the post-processing content of THC-HS was the concentration of RAMEB. Cyclodextrins are known to solubilize lipophilic drugs through inclusion phenomena. The standardized pareto chart and the Main Effects plot (Fig.7-2) clearly illustrate the effect of RAMEB on post-processing content. The content of THC–HS in the films increased with the increasing percentage of RAMEB in films. The coefficient of RAMEB also suggests that it has a positive effect on the content of THC-HS. In a previous communication[197], we had demonstrated an increase in solubility of THC-HS by its inclusion into the hydrophobic cavity of RAMEB thereby rendering it hydrophilic. We also demonstrated that the ester side chain of the prodrug was involved in the hydrogen bonding interactions with the hydroxyl groups on the rim of RAMEB molecule. It is thought that RAMEB increases the stability of THC-HS by encapsulating the molecule in its hydrophobic cavity thus reducing its exposure to heat and oxidation. More specifically, RAMEB significantly ($P < 0.05$) reduces the hydrolysis of THC-HS to THC by protecting its ester linkage through hydrogen bonding interactions. From the *F* ratios in ANOVA table, RAMEB acts synergistically along with citric acid and significantly ($P < 0.05$) increases the post-processing content of THC-HS in the transmucosal films.

From the standardized pareto chart and the Main Effects plot (Fig.7-2), VES did not show a statistically significant effect on the content of THC-HS. The post-processing content of THC-HS did not increase on increasing the concentration of VES in the films. This may be due to the fact it is not essential for actual stability of THC-HS. The definite role of VES in hot-melt cast films is that of plasticizing the polymer PEO N80 by reducing its melt viscosity and ultimately lowering the temperature of casting films. The lower concentrations of VES might just be sufficient to improve the melt viscosity of the polymer. This was also corroborated by the results

obtained by Thumma *et al* in which the stabilization effect of VES was sealed off at 20%w/w in the transmucosal films.[215]

The *F* ratio from ANOVA indicated that VES did not have any statistically significant effect ($P > 0.05$) of post-processing content of THC-HS.

7.4.2. Influence of investigated formulation variables on dissolution profile of THC-HS in the transmucosal films (Y_2)

Table. 3 illustrates the dissolution profiles at the end of 60 minutes for the eleven film formulation prepared for the study. The standardized pareto chart and Main Effects plot for T_{60} % (% drug released at the end of 60 minutes) shown in Fig. 7-3 demonstrate that the concentration of pH modulating agent, citric acid and synthetic modified cyclodextrin, RAMEB had the greatest effect on the dissolution profile and that the effect was statistically significant. All formulations with higher levels of citric acid (F7, F8, F3, F4) exhibited higher % release of THC-HS from the polymeric transmucosal films. From the regression equation for %THC-HS release, it is clear that the coefficient for the independent variable, citric acid is the highest among all the coefficients of other independent variables. From the *F* ratio of ANOVA table, citric acid is the most significant factor affecting the release of THC-HS from the transmucosal films. This can be explained on the basis of stabilization mechanism of THC-HS. By virtue of being able to modulate the microenvironment pH in the transmucosal films, citric acid reduces the hydrolysis rate of hydrophilic prodrug, THC-HS to more hydrophobic water-insoluble parent molecule, THC.

This phenomenon increases the fraction of hydrophilic prodrug THC-HS in the polymeric PEO N80 matrix thereby enhancing its the dissolution rate from the polymeric film.

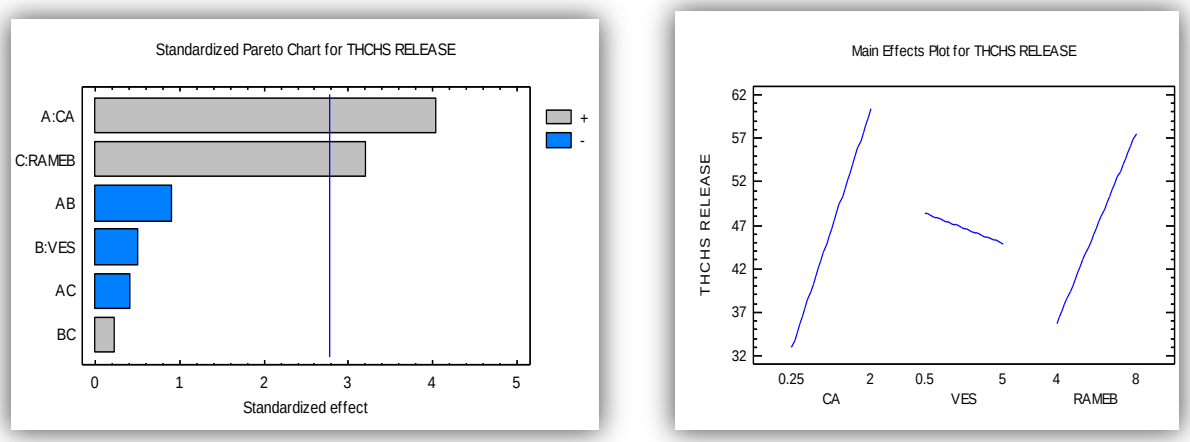

Figure 7-3: Standardized pareto chart and Main effects for post-processing content of THC-HS (Y_2)

The second most significant factor affecting the release of THC-HS from the transmucosal films is RAMEB. RAMEB significantly enhanced the dissolution rate of THC-HS from the films as indicated by *F* ratio ($P < 0.05$) from the ANOVA table. This phenomenon can be explained by the unique capability of RAMEB to solubilize lipophilic water-insoluble drugs through inclusion phenomenon. RAMEB encapsulated the lipophilic portion of THC-HS into its hydrophobic cavity whereas the hemisuccinate ester side chain was protected by hydrogen bonding interactions occurring with hydroxyl groups on the rim of RAMEB molecule. The solubilization of THC-HS by RAMEB increases the hydrophilic fraction of THC-HS in the polymeric matrix thus enhancing the diffusion of the drug once the film is wetted by the dissolution medium.

As expected, VES did have a negative, though not significant influence on the release of THC-HS from the polymeric PEO N80 matrices. At the microenvironmental pH of 4 -5 created by addition of citric acid to the formula, VES was largely present in unionized form, its approximate pka being 4.13.[216] The unionized lipophilic form of VES hindered the dissolution of

THC-HS from the hydrophilic polymeric matrix transmucosal films. The increasing concentrations of VES aggravated the problem further giving rise to the slower release profiles observed in formulations F2 and F4. The negative effect on dissolution profile of THC-HS by VES was not statistically significant as indicated by *F* ratio in ANOVA ($P > 0.05$).

The interactions observed in Fig7-.4. in this case between citric acid as well as VES (AB) though not significant can be explained in terms of presence of insoluble unionized form of VES hindering resistance to the dissolution of more hydrophilic THC-HS.

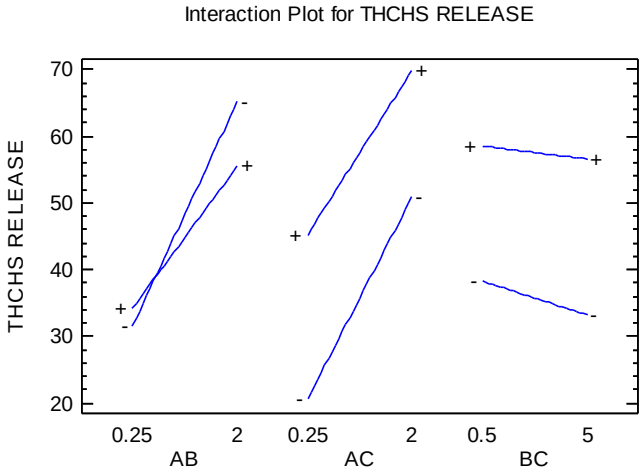

Figure 7-4: Interactions plot for % release of THC-HS at 60 minutes

The value of the correlation coefficient (R^2) of the regression equation for THC-HS release was found to be 0.88, indicating a moderate fit.

The replicated centerpoints in the factorial design provided additional degrees of freedom and made it possible to assess the pure error of the experiments and enabled the model's lack of fit to be checked. In this study, the model was checked for lack of fit for both the responses, post-processing content and % release of THC-HS at 60 minutes. For lack of fit *P* values, we obtained

0.0969 and 0.126 for post-processing content and % release of THC-HS at 60 minutes respectively. Hence the current model provided a satisfactory fit to the data ($P > 0.05$) and had no lack of fit.

7.4.3. Checkpoint Analysis

Three checkpoint batches were prepared and evaluated for post-processing content and release of THC-HS from polymeric matrices at the end of 60 minutes. When measured values were compared with predicted values using Student's *t* test (Table.5), the differences were found to be insignificant ($P > 0.05$). Thus we can conclude that the obtained mathematical equation is valid for predicting the THC-HS content and dissolution from the transmucosal films.

Table 7-5: Checkpoint batches with their Predicted and Measured values of post-processing content and % release of THC-HS at the end of 60 minutes.

Batch Code	A (Citric acid)	B (VES)	C (RAMEB)	THC-HS content (%)		THC-HS release at 60 minutes (T_{60} %)	
				Measured	Predicted	Measured	Predicted
E1	1	-1	1	96.2	98.56	75	73.95
E2	1	1	-1	87.88	89.93	47.98	45.27
E3	1	1	1	98.57	97.38	63.05	65.83

7.5. Conclusion

The polymeric transmucosal films of THC-HS with improved process stability and release characteristics were successfully prepared with the help of the statistical design.

Statistical analyses indicate that pH modulation by citric acid and solubilization with the aid of RAMEB are important factors controlling the stability and release rate of THC-HS from polymeric transmucosal films. Hydrophobic plasticizers such as VES hinder the release of THC-HS from the films. The higher concentrations of citric acid and RAMEB resulted in higher post-processing content and release of THC-HS from the films. All the independent variables studied had significant involvement on the outcome of the dependent variables. The design of experiments methodology assisted in identifying the critical formulation variables in the formulation development of transmucosal films of THC-HS. The encouraging results from the study prompted us to believe that the stable and highly bioavailable transmucosal films of THC can be produced by taking advantage of prodrug solubilization and stabilization strategy. The

reduced number of runs and consolidated information obtained in this study helped in minimizing the amount of drug used in the formulation development of novel hydrophilic prodrug of THC viz., THC-HS.

CHAPTER 8

Preformulation studies of THC-Aminophenylbutyrate (THC-APB)

8.1. Preformulation studies of THC-Aminophenylbutyrate (THC-APB)

THC-Aminophenylbutyrate (THC-APB) was synthesized with an objective of obtaining better stability characteristics than the existing ester prodrugs like THC-HS. Preformulation studies of THC-APB were carried out and include intrinsic solubility, pH solubility, pH stability studies, log p and pKa determination.

8.2. Methods

Intrinsic solubility was determined in water by equilibration for 3 days. pH solubility studies involved a 3-day incubation study of THC-Aminophenyl butyrate (THC-APB) in buffer solutions ranging in pH from 3-9.

Stability of THC-APB in pH 3, 5, 7.4 and 9 -phosphate buffer at 25°C was determined. A prodrug stock solution (100 µL) in ethanol was subsequently added to the 1 ml of buffer solutions to yield a concentration of 10µg/ml. The vials were placed in a constant shaker bath set at 25°C and 60 rpm. Samples (100 µL) were collected at appropriate time intervals for up to 72 hours and stored at –80°C until further analysis. Linear regression of the log concentration versus time profiles yielded the pseudo first order rate constants of degradation.

Feasibility testing of THC-APB for transmucosal delivery was carried out by formulating of hot-melt cast patches. Briefly hot-melt cast patches were prepared using a die and punch. The mixture of hot-melt polymer, excipient and prodrug was heated at 120°C for 5mins and then punched to form a flat circular thin patch. The effect of citric acid on stability of THC-APB hot-melt cast patches was also studied to determine the effect of pH on solid state stability of prodrug in the patch matrix.

8.3. Results and Discussion

8.3.1. Preformulation studies of THC-APB

Table 8-1: Physicochemical properties of THC-APB

Physicochemical properties	
Solubility	15µM
pKa	5,66, 7.47
clogP	7.954
tPSA	61.55

THC-APB was found to exhibit higher aqueous solubility as compared to the parent drug THC (15 µM vs. 2.2 µM). In this prodrug, multiple species with varying solubility are present. Solubility of the prodrug was found to be highest in the acidic pH. Presence of basic groups in the structure resulted in a higher solubility in acidic pH. This also explains a reduced solubility in higher pH.

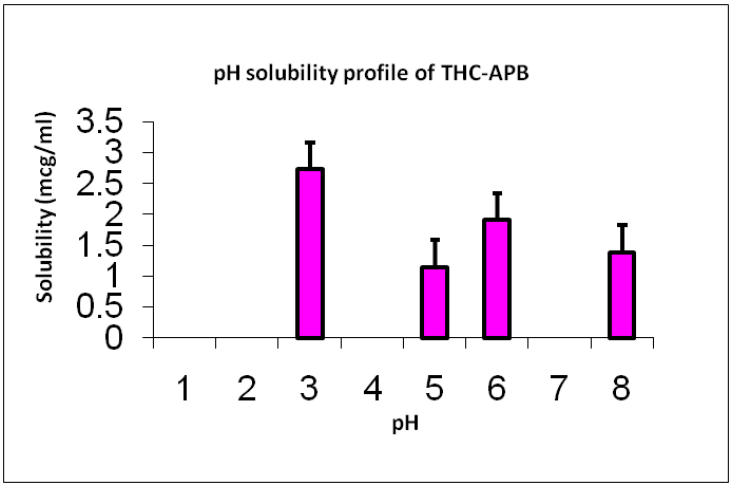

Figure 8-1: pH solubility profile of THC-APB

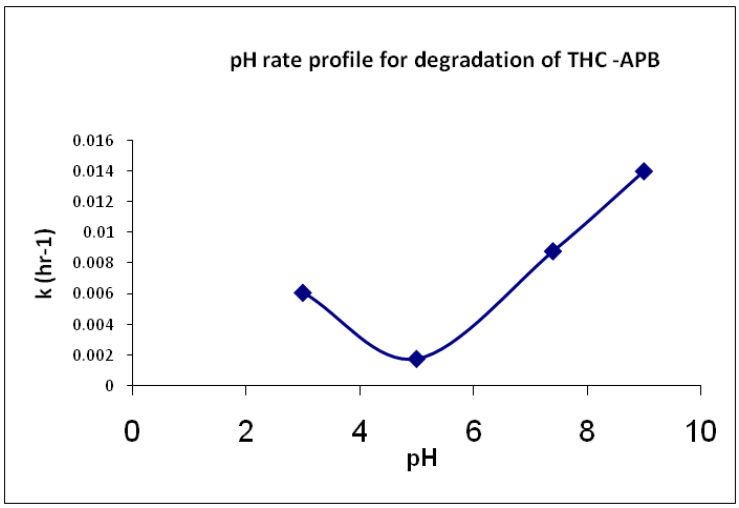

Figure 8-2: pH degradation profile of THC-APB

The hydrolysis followed apparent first order kinetics, and the rate constants (*K*) were obtained as slopes from the semi-logarithmic plots of the unchanged prodrug concentration versus time. The degradation rate constant was found to be the lowest at pH 5 which indicates that the drug is most stable at pH 5 (of those pH tested). Also, the drug undergoes alkaline hydrolysis which was clearly evident from high k values at pH 9.

Table 8-2: Degradation rate constants for THC-APB

pH	k (hr^{-1})	$t_{½}$ (days)
3	**0.0061**	**4.73**
5	**0.0018**	**16.04**
7.4	**0.0088**	**3.28**
9	**0.014**	**2.06**

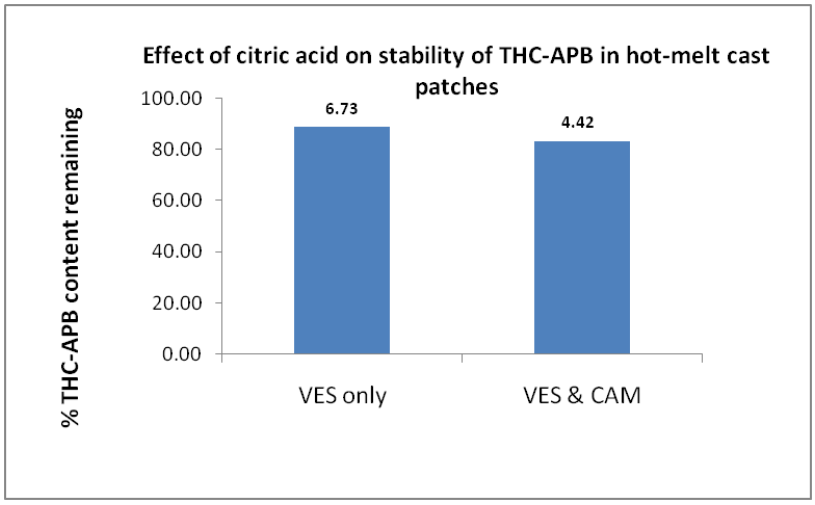

Figure 8-3: Effect of citric acid on stability of THC-APB

Also the content of THC-APB in hot-melt patches containing citric acid is less (83%) as compared to that of the patches devoid of citric acid (89%). This can be attributed to the variable solubility of the ionized and unionized prodrug at acidic pH. THC-APB was found to be reasonably stable at higher casting temperatures than the parent drug, THC.

8.4. Conclusion

Although THC-APB exhibited lower solubility than THC-HS, it was found to have better characteristics as regards to its hydrolytic stability in comparison to THC-HS. When exposed to hot-melt cast temperatures, THC-APB showed significantly higher immediate post-processing content than THC-HS. It is definitely worthwhile exploring the feasibility of developing THC-APB transmucosal films.

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VITA

Born in 1978, Sampada Bhaskar Upadhye received a Bachelor's degree in Pharmacy (B.Pharm. Sci) from SVB College of Pharmacy, Dombivli, State of Maharashtra, India in 2000. Immediately after completing her graduation, she joined the Graduate school at Bombay College of Pharmacy, Mumbai India for a Master's Degree in Pharmaceutical Sciences. Sampada worked as a Graduate research Assistant (08-2001-082002) under the guidance of Dr. Savita R Kulkarni, also her Major Professor at Bombay College of Pharmacy while implementing her research project for a Master's degree. She completed Master's in Pharmaceutical Sciences with emphasis on formulation development of herbal products in August 2002.

She joined the fastest growing contract research firm in India, Rubicon Research Pvt. Ltd, Mumbai in 2002 in the capacity of Research Scientist. At Rubicon Research, she was the team leader for the formulation project from Solvay Pharmaceuticals, Netherlands and worked on two other ANDA projects for European companies for two years.

In 2004, Ms. Sampada B Upadhye commenced the graduate studies for the Doctoral degree at The University of Mississippi. She joined Dr. Michael A. Repka's group in 2005. Since then, she has worked in the capacity of the teaching assistant for the undergraduate courses Phar 331 & 332 as well as the graduate research assistant in Dr.Repka's group. The main focus of Sampada's research in her graduate studies was development of hot-melt extruded transmucosal polymeric films of water-insoluble drugs such as prodrugs of Δ^9-Tetrahydrocannabinol and clotrimazole. She has developed thermally stable and soluble polymeric films of hemisuccinate ester of Δ^9-Tetrahydrocannabinol for transmucosal application to the buccal mucosa.

Sampada was recognized by honor societies like Rho Chi (2007), Sigma Xi (2007) and Who's Who Among Students in American Colleges and Universities (2008) for her academic and scholastic achievements. She has also received a research grant ($1000) for outstanding research proposal from Sigma Xi Research Society. Her grant proposal was selected from numerous other proposals submitted at the national level.

She has received awards for her outstanding research presented in the form of posters and oral talk in Pharmforum regional conferences (2007, 2009) as well as Sigma Xi research conference (2009). She also received "Modified Release Focus Group" Travelship award sponsored by Schering Plough, Inc. for travel to AAPS Annual Meeting and Exposition at Atlanta, GA in 2008. MS. Sampada completed her summer graduate internship at Pfizer, Inc, La Jolla, California where she received a top performance evaluation from her supervisor there.

Ms. Sampada has also offered her services to the University and her professional community, American Association of Pharmaceutical Scientists (AAPS) in the capacity of Director for Graduate Affairs and Vice-chair for AAPS student chapter.

Additionally, Ms. Sampada received a NIH Predoctoral Fellowship sponsored by Center of Biomedical Research and Excellence, The University of Mississippi for consecutively two years (2007-2008 & 2008-2009) for pursuing research in the field of neuroscience.

She has published research as well as review articles as listed below and presented her research in various national and international conferences.

List of Publications

1. MA Repka, S Majumdar, SK Battu, R Srirangam, **SB Upadhye**; (2008) Applications of hot-melt extrusion for drug delivery. Expert Opin. Drug Deliv. 5(12), 1357-1376 ***(Most Downloaded Article in 2009)***

2. **SB Upadhye**, SJ Kulkarni, S Majumdar, MA Avery, W Gul, MA ElSohly, MA Repka; Preparation and characterization of Δ9-Tetrahydrocannabinol hemisuccinate-cyclodextrin complexes ; *AAPS PharmSciTech; 2010, 11(2); 509-517*

3. MA Repka, MM Crowley, S Thumma, **SB Upadhye**, SK Battu, C Martin, JW McGinity, "Hot-Melt Extrusion for Pharmaceutical Applications, A Review: Part I"; **Invited Review**; *Drug Dev. Ind. Pharm*, 33:9, 909-926, 2007

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